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(54) Title: BINDING AGENT

(57) Abstract: This invention relates to agents which bind to cell surface receptors; methods to manufacture said agents; therapeutic compositions comprising said agents; and screening methods to identify novel agents.

BINDING AGENT

FIELD OF THE INVENTION

This invention relates to agents which bind to cell surface receptors; methods to manufacture said agents; therapeutic compositions comprising said agents; and screening methods to identify novel agents.

BACKGROUND OF THE INVENTION

Intercellular and/or intracellular signalling via receptor mediated activation of biochemical and/or molecular mechanisms is a fundamental process for regulating cellular and/or tissue homeostasis. Typically, ligands which interact with receptors to bring about a suitable biochemical response are known as agonists and those that prevent, or hinder, a biochemical response are known as antagonists. For example, and not by way of limitation, cell specific growth factors are ligands that act as agonists and bind receptors located at the cell surface. Activation of the receptors by ligand-specific binding promotes cell proliferation via activation of intracellular signalling cascades that result in the expression of, amongst other things, cell-cycle specific genes, and the activation of quiescent cells to proliferate. Growth factors may also activate cellular differentiation.

A large group of growth factors, referred to as cytokines, are involved in a number of diverse cellular functions. These include, by example and not by way of limitation, modulation of the immune system, regulation of energy metabolism and control of growth and development. Cytokines which are secreted by lymphocytes are termed lymphokines (also known as interleukins). Those secreted by monocytes and macrophages are termed monokines. Cytokines are also secreted by endocrine glands, (for example growth hormone (GH) by the pituitary gland), and adipose cells (for example leptin). Cytokines mediate their effects via receptors expressed at the cell surface on target cells.

Receptors of the cytokine receptor family possess a single transmembrane domain and lack intrinsic enzyme activity (1). Upon the binding of a cytokine to a cognate receptor, either receptor homo- or hetero-dimerisation or oligomerisation occurs. The receptor complex is internalised and signalling occurs through the activation of associated signalling cascades that include the Jak/Stat and Mapk pathways. Internalisation is followed by a recycling step whereby the receptor molecule is regenerated for further use within the cell.

The study of receptor/ligand interactions has been facilitated by the ability to define the structures of receptor molecules and their ligands. Several approaches, including X-ray crystallography and computer modelling, have greatly facilitated our understanding of the biology of ligand: receptor binding.

An example of the above is described with respect to GH and its binding to the growth hormone receptor (GHR). This example is merely meant to be illustrative and not limiting and is an example of a cytokine which activates a signal transduction cascade by binding, dimerisation and internalisation of the receptor:ligand complex.

It is known that a single molecule of growth hormone (GH) associates with two receptor molecules (3-6). This occurs through two unique receptor-binding sites on GH and a common binding pocket on the extracellular domain of two receptors. Site 1 on the GH molecule has a higher affinity than site 2, and receptor dimerization is thought to occur sequentially with one receptor binding to site 1 on GH followed by recruitment of a second receptor to site 2.

The extracellular domain of the GHR exists as two linked domains each of approximately 100 amino acids (SD-100), the C-terminal SD-100 domain being closest to the cell surface and the N-terminal SD-100 domain being furthest away. It is a conformational change in these two domains that occurs on hormone binding with the formation of the trimeric complex GHR-GH-GHR (Fig 5). It has been proposed that

ligand-driven receptor dimerization is the key event leading to signal activation (3), triggering phosphorylation cascades that include the Jak2/Stat5 pathway (7). Using confocal microscopy and Frequency Resonance Energy Transfer (FRET) it is known that there is very rapid internalisation of GHR after ligand binding and that internalisation and signalling are independent functions (16). Internalisation of the GHR-GH-GHR complex is followed by a recycling step whereby the receptor molecule is regenerated for further use within the cell.

The importance of receptor dimerization in signal transduction is indicated by a number of experiments. High concentrations of GH, which favour the monomeric GH-GHR complex, inhibit the GH signal (8). Mutations in the inter-receptor dimerization domain inhibit signalling without influencing GH binding (10). The strongest evidence comes from work with a GH molecule mutated at site 2 to prevent receptor dimerisation. These GH mutants block GH-stimulated cell proliferation (8,11-14), the conformational change associated with receptor dimerization (15), and Jak-Stat signalling (16).

US 5,849,535 describes a human growth hormone including a number of amino acid substitutions which disrupt Site 2 binding. The substitution of a different amino acid at G120 is one modification that disrupts Site 2 binding and the hGH variant acts as an hGH antagonist.

The in vivo efficacy of hGH and hGH variants is determined, in part, by their affinity for the hGH receptor and rate of clearance from the circulation. The kidneys are relatively small organs which receive approximately 25% of cardiac output. The kidneys perform several important functions primarily related to the regulation of the composition and volume of body fluids. The kidneys filter about 100 litres of plasma every day and of the blood flow in and out of a kidney only approximately 1% becomes urine. Approximately 20% of the plasma that passes through the kidney gets filtered into the nephron. Filtration takes place in the glomerulus which is driven by the hydrostatic pressure of the

blood. Water and small molecules are filtered whereas blood cells and large molecules, for example polypeptides, do not pass through the glomerular filter.

Those polypeptides with an effective molecular weight above 70 kDa are not cleared by glomerular filtration because they are simply too large to be filtered. Certain proteins of small molecular weight are filtered by the glomerulus and are found in the urine. For example, Growth Hormone (GH has a molecular weight of 22.1 kDa and the kidney is responsible for clearing up to 60-70% of GH in humans (Baumann, 1991; Haffner et al, 1994), and up to 67% in rat (Johnson & Maack, 1977). Other examples of relatively small molecular weight polypeptides which are filtered by the kidney include leptin, erythropoeitin, and IL-6.

Syed *et al* (1997) constructed an anti-coagulant fusion protein which fused hirudin with albumin. This fusion protein showed extended plasma half life whilst maintaining a potent anti-thrombin (anti-coagulant) activity. This is likely to result from decrease in glomerular filtration by the kidneys. However a problem associated with this strategy is that hirudin is a foreign protein and which is known to provoke a strong immune response. The increase in molecular weight of the hirudin fusion protein increases the catabolic half-life from 0.7 hours to 4.6 days.

A further method to increase the effective molecular weight of proteins and to produce a product which has reduced immunogenicity is to coat the protein in polyethylene glycol (PEG). The *in-vivo* half-life of GH has been increased by conjugating the proteins with poly ethylene glycol, which is termed "pegylation" (See Abuchowski et al., *J.Biol Chem.*, 252:3582-3586 (1977). PEG is typically characterised as a non-immunogenic uncharged polymer with three water molecules per ethylene oxide monomer. PEG is believed to slow renal clearance by providing increased hydrodynamic volume in pegylated proteins (Maxfield et al., *Polymer*, 16:505-509 (1975)). US 5,849,535 also describes humanGH (hGH) variants which are conjugated to one or more polyols, such as poly(ethylene glycol) (PEG).

An alternative to pegylation which provides a molecule which retains biological activity and is immune silent is herein disclosed. A chimeric protein comprising the extracelluar domain, or part thereof, of a receptor linked, via a variable flexible linker molecule to its cognate ligand to produce an agent with an apparent molecular weight greater than the native ligand. In the example provided, GH is fused to at least part of the growth hormone receptor (GHR) which gives an approximate molecular weight of 55kDa which when glycosylated increases the effective molecular weight to approximately 75kDa. This would be of sufficient size to prevent the chimera being filtered by the kidney and, importantly, the molecule retains biological activity.

A long-acting form of growth hormone could be used in the treatment of both childhood and adult onset growth hormone deficiency. Growth hormone has well known anabolic actions and a long-acting form of growth hormone could be used for the treatment of a number of conditions by virtue of its anabolic actions including promoting growth in Turner's syndrome, renal failure, osteoporosis and muscle wasting in catabolic patients. Bovine somatotropin is currently used to enhance milk production from cows. A long-acting form of growth hormone would be an effective treatment for increasing milk yield from cows (Peel et al. 1981).

This strategy is applicable to other ligand-receptor combinations (eg. leptin, erythropoeitin and IL-6). For example, leptin is being trialed as a therapy for obesity (Mantzoros & Flier, 2000). A long-acting form of leptin could be used to treat obesity, insulin resistance, hyperlipidaemia and hypertension. Erythropoeitin is important in the generation of red cell mass. A long-acting form of erythropoeitin could be used to treat anaemia especially that associated with renal failure.

Truncated GH receptors, which lack the cytoplasmic domain of the receptor, act as dominant negative inhibitors of GH signalling (9,19). The truncated receptor is expressed at a high level on the cell surface because it lacks the cytoplasmic domain essential for internalisation (16). In the presence of GH, the truncated receptor

heterodimerises with the full length receptor and blocks signalling because it lacks the cytoplasmic domain. As the truncated receptor fails to internalise it acts as a dominant negative inhibitor preventing internalisation of the GH receptor complex.

The disorders of acromegaly and gigantism result from an excess of growth hormone, usually due to pituitary tumours. A drug currently under trial is the pegylated GH antagonist B2036, designed using recently acquired knowledge of the molecular structure of the growth hormone receptor (GHR). Unfortunately, high levels of B2036 are required to antagonise GH action with drug levels over a 1000 times higher than endogenous GH levels (18).

B2036, despite having a mutated site 2, binds to a receptor dimer, and is internalised in an identical fashion to GH. It does not however trigger the conformational change required for signalling. The high dose requirement of the antagonist relates to its internalisation and its differential binding to soluble and membrane bound receptor. The pegylated antagonist does not bind efficiently to membrane bound receptor although pegylation increases half-life and lowers immunogenicity. The non-pegylated antagonist is rapidly internalised and cleared.

There is a need to provide an antagonist that is not internalised by the cell and that can be delivered in lower doses. This would prove a more effective and potent antagonist and provide a more effective and economical treatment.

The leptin receptor (28) and erythropoietin (EPO) receptor (29,30) share considerable structural homology to the GHR and require a similar dimerisation process to trigger signalling. Leptin supresses appetite and leptin resistance is associated with obesity. A leptin receptor antagonist will provide a treatment for anorexia nervosa. EPO excess causes polycythaemia which may be secondary to hypoxia (chronic lung disease), or primary in the case of polycythaemia rubra vera (a disorder of excess red blood cells). An EPO antagonist will provide a therapy for polycythaemia.

A further example of a receptor: ligand binding is provided by the IL-6 activation of its cognate receptor. The current model for IL-6 activation of its cognate receptor stipulates that IL-6 binds to either soluble or membrane bound IL-6 receptor (IL-6R). The IL-6 / IL-6R complex then recruits two molecules of gp130 and the tetramer signals through the dimerisation of the two gp130 molecules which possess cytoplasmic domains that associate with signalling molecules (Grotzinger *et al.*, 1999). In nature IL-6 and the IL-6R exist as separate molecules which possess high affinity binding sites for each other and the association with the signal transducing molecule gp130 occurs through covalent linkage and the formation of disulfide bonds.

We have been studying receptor trafficking and binding protein production for two members of the cytokine receptor family; GH and leptin (9,16,20,21). These two hormones play a fundamental role in determining body composition in adults. Both leptin and GH are important in regulating energy expenditure, appetite, and fat mass. The ability to manipulate the biological actions of leptin and GH will have important therapeutic outcomes for the treatment of both hormone excess and deficiency.

Using confocal microscopy and Frequency Resonance Energy Transfer (FRET) we have shown that there is very rapid internalisation of GH receptor after ligand binding and that internalisation and signalling are independent functions (16). Our recent work shows that the GH antagonist, pegvisomant, despite having a mutated site 2, binds to a receptor dimer, is internalised in an identical fashion to GH, but does not trigger the conformational change required for signalling. We have demonstrated that the high dose requirement of the antagonist relates to its internalisation and its differential binding to soluble and membrane bound receptor. The pegylated antagonist does not bind efficiently to membrane bound receptor and the non-pegylated antagonist is rapidly internalised and cleared.

We demonstrate that a truncated GHR, which lacks the cytoplasmic domain of the receptor, can act as a dominant negative antagonist of GH signalling, (Figure 5) (9,20). The truncated receptor is expressed at a high level on the cell surface as it lacks cytoplasmic domain essential for internalisation (16). The truncated receptor heterodimerises with the full length receptor, blocks signalling as it lacks the cytoplasmic domain, and acts as a dominant negative because it is present in excess on the cell surface and prevents internalisation of the GH receptor complex.

There are two problems associated with using truncated receptors in the generation of antagonists to GH. A truncated receptor in the membrane would have to be generated from within the cell. The GHR is also proteolytically cleaved and in time the majority of the truncated receptor would be lost into the circulation.

We link GH, through its C-terminus and a linker, to the N-terminus of the C-terminal SD-100 domain of the GHR. By varying the length of the linker we define a molecule that has the flexibility to allow binding of GH through site 1 to full length receptor at the cell surface. The C-terminal SD-100 domain of the receptor will then rotate in to complete the trimeric structure GHR-GH-GHRtr where GHRtr is the C-terminal SD-100 domain. This complex neither signals nor internalises, and effectively antagonises GH action. It has the additional advantages of low immunogenicity and low clearance as the majority of GH is cleared via the GHR (22).

We also demonstrate that the leptin receptor produces a soluble binding protein (21) as do many cytokine receptors (2), and the predominant peripheral form of the leptin receptor is a truncated receptor similar to the truncated GHR (27,28). Our recent work has demonstrated that truncated leptin receptors can inhibit leptin signalling. The erythropoietin (EPO) receptor shares a very similar crystal structure to GHR and an EPO chimera with the C-terminal SD100 of the EPO receptor would function as an antagonist.

STATEMENTS OF INVENTION

According to the present invention there is provided a binding agent comprising a first part capable of binding a ligand binding domain of a receptor linked to a second part comprising a receptor binding domain wherein said binding agent modulates the activity of the receptor.

In one embodiment of the invention, the binding agent antagonises the activity of the receptor.

In an alternative embodiment of the invention, the binding agent agonises the activity of the receptor.

Preferably the first part comprises a cytokine or the binding domain of a cytokine.

More preferably still the first part comprises a cytokine or the binding domain of a cytokine selected from the following: growth hormone; leptin; erythropoietin; prolactin; TNF, interleukins (IL), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11; the p35 subunit of IL-12, IL-13, IL-15; granulocyte colony stimulating factor (G-CSF); granulocyte macrophage colony stimulating factor (GM-CSF); ciliary neurotrophic factor (CNTF); cardiotrophin-1 (CT-1); leukemia inhibitory factor (LIF); oncostatin M (OSM); interferon, IFNα and IFNγ.

Preferably the second part of the binding agent comprises at least part of the cognate receptor of the cytokine or a part of an associated receptor.

Preferably the first part is GH.

Preferably the second part is one extracellular domain of GHR. More preferably the second part is the C-terminal SD-100 domain of GHR.

In an alternative embodiment the first part is IL-6 or a binding domain of IL-6 and the second part is a part ofgp 130.

An embodiment of the invention exploits the high affinity of a cytokine for its receptor and the failure of truncated receptors to internalise to generate a specific receptor antagonist which is a chimera of the cytokine and its cognate receptor. The binding agent of the invention has the important advantage that binding of the cytokine to its receptor does not trigger internalisation of the receptor-cytokine complex. This means that dosage of the antagonist can be minimised.

In one embodiment of the invention, the binding agent is a fusion protein.

In an alternative embodiment of the invention, the first part is linked by a linker to the second part. The linker may be flexible.

The linker could be at any residue within the extracellular domain of the receptor which would allow growth hormone to flexibly bind with the free receptor at the cell surface. Where the first part is GH and the second part is one extracellular domain of GHR, the linkage may be made between any peptide residue in the GH and GHR. Preferably the linkage is made between a residue close to the C-terminus of the GH molecule and a residue close to the N-terminus of the GHR. More preferably the linkage is made between a residue close to the C-terminus of the GH molecule and a residue close to the N-terminal of the N-terminal of the C-terminal SD-100. More preferably the linkage is made at any of residues 126-128 of the N-terminus of the C-terminal SD-100 of the GHR. In one embodiment of the invention, the linkage is made at residue 127 of the N-terminus of the C-terminal SD-100. Preferably the linker is a peptide.

It will be apparent to one skilled in the art that alternative linkers can be used to link first and second parts. By way of example and by no means of limitation, suitable linkers might be a nucleic acid (eg oligonucleotide); a peptide nucleic acid; a chemical crosslinker (eg polyoxyethlene).

The crystal structure of the GHR-GH-GHR complex reveals that the distance between the C-terminus of GH (residue 191) and N-terminus of the C-terminus SD-100 (residue 126-128) is 10A. This provides invaluable information with respect to linker design.

Preferably the linker is a polypeptide which comprises 5 to 30 amino acid residues. More preferably the linker comprises 10 to 20 amino acid residues.

More preferably the linker comprises at least one copy of the peptide:

Gly Gly Gly Gly Ser (hereinafter referred to as "Gly4Ser").

In one embodiment of the invention the linker is 10 amino acids in length and comprises two copies of the Gly4Ser linker. In an alternative embodiment of the invention, the linker is 15 amino acids in length and comprises three copies of the Gly4Ser linker. In yet an alternative embodiment, the linker is 20 amino acids in length and comprises four copies of the Gly4Ser linker.

According to a further aspect of the invention there is provided a nucleic acid molecule comprising a nucleic acid sequence which encodes a binding agent according to the invention selected from the group consisting of:

i) the group comprising Figs 4, 5, 8, 9 and 21;

ii) nucleic acids which hybridise to the sequences of (i) above and which have receptor modulating activity; and

iii) nucleic acid sequences which are degenerate as a result of the genetic code to the sequences defined in (i) and (ii) above.

In a preferred embodiment of the invention said nucleic acid hybridises under stringent hybridisation conditions to the sequences represented in Figs 4,5,8,9, or 21.

Stringent hybridisation/washing conditions are well known in the art. For example, nucleic acid hybrids that are stable after washing in 0.1x SSC,0.1% SDS at 60°C. It is well known in the art that optimal hybridisation conditions can be calculated if the sequence of the nucleic acid is known. For example, hybridisation conditions can be determined by the GC content of the nucleic acid subject to hybridisation. Please see Sambrook et al (1989) Molecular Cloning; A Laboratory Approach. A common formula for calculating the stringency conditions required to achieve hybridisation between nucleic acid molecules of a specified homology is:

$T_m = 81.5^0\ C + 16.6\ Log\ [Na^+] + 0.41[\ \%\ G + C] - 0.63\ (\%formamide).$

Typically, hybridisation conditions uses 4 – 6 x SSPE (20x SSPE contains 175.3g NaCl, 88.2g $NaH_2PO_4\ H_2O$ and 7.4g EDTA dissolved to 1 litre and the pH adjusted to 7.4); 5-10x Denhardts solution (50x Denhardts solution contains 5g Ficoll (type 400, Pharmacia), 5g polyvinylpyrrolidone abd 5g bovine serum albumen/500ml; 100µg-1.0mg/ml sonicated salmon/herring DNA; 0.1-1.0% sodium dodecyl sulphate; optionally 40-60% deionised formamide. Hybridisation temperature will vary depending on the GC content of the nucleic acid target sequence but will typically be between 42^0- 65^0 C.

According to a further aspect of the invention there is provided a polypeptide which is encoded by a nucleic acid molecule according to the invention.

In a preferred embodiment of the invention the polypeptide so encoded is modified by deletion, addition or substitution of at least one amino acid residue. Ideally said modification enhances the antagonistic or agonistic effects of said polypeptide with respect to the inhibition or activation of receptor mediated cell signalling.

Alternatively, or preferably, said modification includes the use of modified amino acids in the production of recombinant or synthetic forms of polypeptides.

It will be apparent to one skilled in the art that modified amino acids include, by way of example and not by way of limitation, 4-hydroxyproline, 5-hydroxylysine, N^6-acetyllysine, N^6-methyllysine, N^6,N^6-dimethyllysine, N^6,N^6,N^6-trimethyllysine, cyclohexyalanine, D-amino acids, ornithine. The incorporation of modified amino acids may confer advantageous properties on polypeptides comprising Fig 21. For example, the incorporation of modified amino acids may increase the affinity of the polypeptide for its binding site, or the modified amino acids may confer increased *in vivo* stability on the polypeptide thus allowing a decrease in the effective amount of therapeutic polypeptide administered to a patient.

According to a yet further aspect of the invention there is provided a vector including a DNA molecule encoding a binding agent according to any preceding aspect or embodiment of the invention.

In a preferred embodiment of the invention said vector is provided with means to recombinantly manufacture the binding agent of the invention.

In a preferred embodiment of the invention said vector is an expression vector adapted for prokaryotic gene expression.

Prokaryotic expression systems are well known in the art and comprise vectors adapted for high level constitutive and inducible expression. Inducible expression systems are particularly advantageous if the recombinant polypeptide is toxic to the bacterial cell. Induction of expression is tightly regulated by promoters responsive to various inducers (eg IPTG inducible). Bacterial cells can be grown to stationary phase before induction thereby reducing harmful effects of toxic polypeptides.

Additionally it is also well known in the art that certain polypeptides are difficult to manufacture recombinantly due, for example, to protein instability or problems of aggregation. It is well known that genetically modified bacterial strains are available which are mutated in genes (eg bacterial proteases) which facilitate the production of native and recombinant bacterial polypeptides.

In a further preferred embodiment of the invention said vector is an expression vector adapted for eukaryotic gene expression.

Typically said adaptation includes, by example and not by way of limitation, the provision of transcription control sequences (promoter sequences) which mediate cell/tissue specific expression. These promoter sequences may be cell/tissue specific, inducible or constitutive.

Promoter is an art recognised term and, for the sake of clarity, includes the following features which are provided by example only, and not by way of limitation. Enhancer elements are *cis* acting nucleic acid sequences often found 5' to the transcription initiation site of a gene (enhancers can also be found 3' to a gene sequence or even located in intronic sequences and are therefore position independent). Enhancers function to increase the rate of transcription of the gene to which the enhancer is linked. Enhancer activity is responsive to *trans* acting transcription factors (polypeptides) which have been shown to bind specifically to enhancer elements. The binding/activity of transcription factors (please see Eukaryotic Transcription Factors, by David S Latchman, Academic Press Ltd, San Diego) is responsive to a number of environmental cues which include, by example and not by way of limitation, intermediary metabolites (eg glucose, lipids), environmental effectors (eg light, heat).

Promoter elements also include so called TATA box and RNA polymerase initiation selection (RIS) sequences which function to select a site of transcription initiation. These

sequences also bind polypeptides which function, *inter alia*, to facilitate transcription initiation selection by RNA polymerase.

Adaptations also include the provision of selectable markers and autonomous replication sequences which both facilitate the maintenance of said vector in either the eukaryotic cell or prokaryotic host. Vectors which are maintained autonomously are referred to as episomal vectors.

Adaptations which facilitate the expression of vector encoded genes include the provision of transcription termination/polyadenylation sequences. This also includes the provision of internal ribosome entry sites (IRES) which function to maximise expression of vector encoded genes arranged in bicistronic or multi-cistronic expression cassettes.

These adaptations are well known in the art. There is a significant amount of published literature with respect to expression vector construction and recombinant DNA techniques in general. Please see, Sambrook et al (1989) Molecular Cloning: A Laboratory Manual, Cold Spring Harbour Laboratory, Cold Spring Harbour, NY and references therein; Marston, F (1987) DNA Cloning Techniques: A Practical Approach Vol III IRL Press, Oxford UK; DNA Cloning: F M Ausubel et al, Current Protocols in Molecular Biology, John Wiley & Sons, Inc.(1994).

In yet a further aspect of the invention there is provided a method to prepare a binding agent polypeptide according to the invention comprising:

(i) growing a cell transformed or transfected with a vector or nucleic acid of the present invention in conditions conducive to the manufacture of said polypeptide; and

(ii) purifying said polypeptide from said cell, or its growth environment.

In a preferred method of the invention said vector encodes, and thus said recombinant polypeptide is provided with, a secretion signal to facilitate purification of said binding agent polypeptide.

In yet a further aspect of the invention there is provided a cell transformed/transfected with the vector or nucleic acid according to the invention.

Preferably said cell eukaryotic and is selected from: fungal; insect (eg *Spodoptera frugiperda*); amphibian; plant; mammalian.

More preferably said cell is prokaryotic and is an *E. coli* cell.

Preferably the binding agent of the present invention is used for the manufacture of a medicament for use in the treatment of acromegaly; gigantism; growth hormone deficiency, Turners syndrome; renal failure; osteoporosis, diabetes mellitus, cancer; obesity; insulin resistance; hyperlipidaemia; hypertension (leptin chimera); anaemia; autoimmune and infectious disease; inflammatory disorders including rheumatoid arthritis.

According to a further aspect of the invention there is provided a pharmaceutical composition comprising the binding agent according to the invention. Preferably said pharmaceutical composition includes a carrier, excipient and/or a diluent.

The invention also provides for a method of treating a human or animal subject comprising administering an effective amount of the pharmaceutical composition/medicament to said subject.

It will be apparent to one skilled in the art that the compositions/medicaments can be provided in the form of an oral or nasal spray, an aerosol, suspension, emulsion, and/or eye drop fluid. Alternatively the medicament may be provided in tablet form. Alternative delivery means include inhalers or nebulisers.

Alternatively or preferably the medicament can be delivered by direct injection. It is also envisioned that the compositions/medicaments be delivered intravenously, intramuscularly, subcutaneously or topically. Further still, the compositions/medicaments may be taken orally or rectally.

The invention also provides a method of reduced renal clearance of a molecule comprising forming a binding agent according to any embodiment of the invention

An embodiment of the invention will now be described by example only and with reference to the following figures wherein;

Figure 1 represents a schematic diagram of (a) pTrcHis-TOPO and its derivatives; (b) pTrcHis–TOPO/GHstop;(c)pTrcHis-TOPO/Ghstop/GHR;(d)pTrcHis-TOPO/GH/link/GHR; (e) pTrcHis-TOPO/GH/link/flecGHRstop; f) pJONEXGHstop; pJONEXGHstoplink GHR;

Figure 2 represents the sequence of the cDNA of the 588bp PCR amplified GH fragment;(The 3'- Not1 site and two stop codons are shown in bold and italics respectively)

Figure 3 represents the sequence of the cDNA of the 390bp PCR amplified GHR SD 100 fragment (The 5' EcoRI and 3' HindIII restriction sites are shown in bold and the 3' stop codons are shown in italics);

Figure 4 represents the nucleic acid sequence of the full length GHstopGHR SD100 construct;

Figure 5 represents the nucleic acid sequence of the full length GHlinkGHR construct (Not1, EcoRI and HindIII restriction sites are shown in bold and the two 3' stop codons are shown in italics);

Figure 6: represents the protein sequence of full length GHlinkGHR (340 amino acids);

Figure 7 represents the nucleic acid sequence of the 762bp PCR amplified full length extracellular domain of GHR (GHRflec) (the 5' EcoRI and HindIII sites are shown in bold and the two 3' stop codons are shown in italics);

Figure 8: represents the full length nucleic acid sequence of the GHlinkGHRflec construct (the Not1, EcoRI and HindIII site are shown in bold and the two 3' stop codons are shown in italics);

Figure 9: represents the nucleic acid sequence of the 1157bp PCR fragment,GHlinkGHR generated by oligonucleotides TrcRBSsacF and GHRA835H, (the SacI, Not1, EcoRI and HindIII sites are shown in bold, the new ribosome binding site is shown in bold and underlined and the start/stop codons are shown in italics);

Figure 10: represents the nucleic acid sequence of the 740bp PCR fragment, GHstop generated by nucleotides pTrcRBSsacI and TrcHindrev, (The SacI, Not1, EcoRI and HindIII sites are shown in bold, the new ribosome binding site is shown in bold and underlined and the start/stop codons are shown in italics);

Figure 11: represents the complete amino acid sequence of the GH/GHR receptor chimera (GH residues 1-191/ linker /GHR residues 127-246).

Figure 12A represents the full length nucleic acid sequence of IL-6; Figure 12B represents the amino acid sequence of IL-6;

Figure 12 represents the full length nucleic acid sequence of gp130;

Figure 13 represents the amino acid sequence of the IL-6 /gp130 fusion polypeptide;

Figure 14 represents the nucleic acid sequence of the gp 130 domain 1 deletion (616-2112bp);

Figure 15 represents the nucleic acid sequence of gp130 domain 922-2112bp;

Figure 16 represents a western blot of induced proteins expressed by *E. coli* transformed with various vectors;

Figure 17 (a) is a graphical representation of reporter gene assays for Ghstop and GH link GHR; and (b) quantification of the data represented in (a); and

Figure 18 is a schematic representation of GH:GHR interaction and GH:GHR chimera interaction with GHR.

Figure 19 represents the *in vitro* agonist activity of the GH/GHR chimera.

Figure 20 shows the results of a bioassay comparing the induction of a Stat5 reporter (luciferase activity) by growth hormone (GH), a negative control (XL blue) and partially purified antagonist (Chimera 1A2)

Figure 21 represents the nucleotide sequence of the Chi 1A2 chimera.

Figure 22 represents the protein sequence of Chi 1A2 chimera (311 amino acids).

MATERIALS AND METHODS

Table 3 explains the nomenclature used to define the protein constructs.

Generation of GH:GHR Fusion Protein

Six constructs are cloned (including 3 different lengths of linker with or without the C241 of the GHR) into a C-terminal poly-His expression vector. Human GH is amplified using high fidelity proof reading Pfu with convenient restriction sites to clone into the

vector. The C-terminus SD-100 GHR is similarly amplified and the linker constructed in the primer with convenient restriction sites to clone into the C-terminus of GH. The constructs are then fully sequenced.

From the crystal structure of the GHR-GH-GHR complex, the distance between the C-terminus of GH (residue 191) and N-terminus of the C-Terminus SD-100 GHR (residue 126-128) is 10 A. Linkers between 10-20 residues are designed and three constructs made with linkers of either 10, 15 or 20 residues comprising of 2, 3 or 4 copies of the basic Gly_4Ser linker.

Protein Purification

The constructs are expressed in *E. coli* (JM109) and the protein purified on Invitrogen Xpress System Nickel columns with a secondary purification step by ion exchange chromatography. Lipopolysaccharide should not interfere with the bioassay as this requires a relatively short incubation in the cell culture system. If required the chimera antagonist is further purified using polymyxin B columns (Pierce).

Screening of Antagonist Activity

An established bioassay is used to screen for antagonist activity (9). A permanent cell line expressing the full length GHR is transiently transfected with a luciferase reporter that binds activated Stat5 (9). Twenty-four hours later the cells are stimulated with GH for 6 hours with or without antagonist. The cells are then lysed and luciferase activity measured (9).

Screening of Agonist Activity

A permanent cell line expressing the full length GHR is transiently transfected with a luciferase reporter that binds activated Stat5 (9). Twenty-four hours later the cells are stimulated with or without the GH/GHR chimera for 6 hours. The cells are then lysed and luciferase activity measured (9).

PCR OF PITUITARY GH FROM PITUITARY cDNA TO GENERATE GHstop

Full-length human growth hormone was amplified from human pituitary cDNA using the Boehringer Expand High Fidelity PCR System. Each reaction consisted of: Primers GHS1-23 (forward) and GHA573not (reverse) 10μM each, 200μM dNTPs, 5μl Expand buffer plus magnesium chloride (1.5mM), and 0.6μl High fidelity enzyme mix in a total volume of 50μl.

Samples were as follows

1. Pituitary cDNA using GHS1-23 and GHA573not primers
2. Pituitary cDNA using actin specific primers
3. Control cDNA for actin
4. Water control.

PCR reaction master mix 1.

cDNA	2μl pituitary cDNA	2μl pituitary cDNA	2μl control cDNA	2μl water
Forward primer (10μM stock)	2μl **GHS1-23**	Actin primer 1μl	Actin primer 1μl	2μl **GHS1-23**
Reverse primer (10μM stock)	2μl ***GHA573not***	Actin primer 1μl	Actin primer 1μl	2μl ***GHA573not***
dNTP (10mM stock)	2μl	2μl	2μl	2μl
Sterile water	17μl	19μl	19μl	17μl

Master Mix 2 (per reaction)

10 x Expand High Fidelity buffer (plus magnesium) (5µl)
Sterile distilled water (19.4µl)
Expand High Fidelity Expand polymerase (0.6µl)

Added 25µl Master Mix 2 to Master mix 1 and overlaid with mineral oil.

PCR was carried out to the following method:

94°C: 2 minutes,
94°C: 30sec / 54°C: 1 minute / 72°C: 1 minute, for 30 cycles
72°C: 10min.

The 5'-nucleotide (GHS1-23) has sequence homology to the 5' end of the growth hormone gene and the 3'-nucleotide (GHA573not) contains a *Not* I site together with two stop codons. The PCR reaction produced a band of 588bp (see Figure 2) containing full-length human growth hormone. The fragment was then purified using the QIAquick PCR purification kit (Qiagen) and subsequently TOPO cloned into the pTrcHis-TOPO vector (Invitrogen, see Figure 1). Ligations were transformed in to *E. coli* TOPO one shot cells (Invitrogen) by the calcium chloride method. Plasmid mini preparations were produced from positive transformants and screened by restriction digest using *PstI*/*Eco*RI. Clones with the correct insert size were then sequenced using vector specific primers supplied by invitrogen that bind 5' and 3' to the insert region (Xpress forward primer and pTrcHis reverse primer, see Table 1). This construct was named pTrcHisGHstop and was used as the template for subsequent cloning reactions.

Forward primer for Growth hormone primer "GHS1-23":

5'ttcccaaccattcccttatccag 3'

Reverse primer *GHA573not*

5'ttatcagcggccgccgaagccacagctgccctccac 3'

PCR of GHR C-terminal SD100 domain from human liver cDNA

The GHR C-terminal SD100 domain (Figure 3) was amplified from human liver cDNA using the same PCR method as previously described, but using primers GHRS476 (forward) and GHRA835H (reverse), see table 1. The 5'-nucleotide contains an *Eco*RI site whilst the 3'-nucleotide contains two stop codons and a *Hind*III site.
The PCR reaction was carried out and cleaned up as described previously.

Samples were as follows:

1. liver cDNA using GHR476 and GHRA835H
2. liver cDNA using actin specific primers
3. Control cDNA
4. Water control.

PCR reaction: Master Mix 1

cDNA	1µl liver cDNA	1µl liver cDNA	1µl control cDNA	1µl Sterile water
Forward primer (10µM stock)	2µl **GHRS476**	Actin primer 1µl	Actin primer 1µl	2µl **GHRS476**
Reverse primer (10µM stock)	2µl ***GHRA835H***	Actin primer 1µl	Actin primer 1µl	2µl ***GHRA835H***

DNTP (10mM stock)	2µl	2µl	2µl	2µl
Sterile Water	18µl	19µl	19µl	17µl

Master Mix 2 (per reaction)

10 x Expand High Fidelity buffer (plus MgCl2) (5µl)
Sterile distilled water (19.4µl)
High Fidelity Expand polymerase (0.6µl)

Added 25µl Master Mix 2 to Master mix 1 and overlaid with mineral oil.

Both vector, pTrcHisGHstop, and PCR product were subjected to a double digest using *Eco*RI and *Hind*III restriction enzymes (Promega). The PCR product was cleaned up using QIAquick PCR purification kit and the digested pTrcHisGHstop vector was separated by agarose gel electrophoresis and purified using the QIAquick gel extraction kit. The digested PCR fragment containing the C-terminal SD100 domain of GHR was then ligated to the above digested vector and transformed in to TOPO one shot cells (invitrogen) by the calcium chloride method. Ligations were transformed in to *E. coli* TOPO one shot cells (Invitrogen). Plasmid mini preparations were produced from positive transformants and screened by restriction digest using BamHI/*Eco*RI (Promega) and by PCR screening using GHS1-23 and GHRA835H primers. Clones with the correct insert size were then sequenced using pTrcHis reverse and GHseqF primers (see Table 1). This vector was called pTrcHisGHstopGHR and was used as the vehicle for the insertion of linker regions of varying lengths between GH and GHR in to the Not1/ EcoRI sites. Figure 4 shows the full insert sequence for pTrcHisGHstopGHR.

This construct allows the insertion of a linker molecule in to the Not1/EcoRI sites between Ghstop and GHlinkGHR.

Insertion of linker regions

The initial linker was constructed composed of a 4x repeating sequence of four glycine residues and one serine residue (20 residues in total) by annealing oligonucleotides G4S4 (forward) and G4COM4 (reverse) see Table 1. The 5'-nucleotide contains a *Not*I site and the 3'-nucleotide contains an *Eco*RI site. The vector pTrcHisGHstopGHR, was double digested with Not1 and HindIII restriction enzymes and cleaned up using the QIAquick clean up kit (Qiagen).

"G4S4"

5'ggccgcggtggcggaggtagtggtggcggaggtagcggtggcggaggttctggtggcggaggttccg 3'

"G4COMS4"

5' aattcggaacctccgccaccagaacctccgccaccgctacctccgccaccactacctccgccaccgc 3'

Preparation of linker insert:

Oligonucleotides G4S4 and G4COMS4 were resuspended in annealing buffer [10mM Tris-HCl, pH 7.5, 50mM NaCl, 1mM EDTA] to a final concentration 0.1pmol/µl. An equal volume of each oligonucleotide was then mixed and heated to 95°C for 2 minutes and then allowed to cool over a 1hour period.

The oligonucleotide duplexes were then ligated to the Not1/EcoRI double digested vector pTrcHisGHstopGHR and transformed in to TOPO one shot cells (Invitrogen) by the calcium chloride method. Plasmid mini preparations were produced from positive transformants and screened by restriction digest using Not1/*Eco*RI and by PCR screening using GHS1-23 and GHRA835H primers. Clones with the correct insert size

were then sequenced using pTrcHis reverse primer and GHseqF (see Table 1). This vector was called pTrcHisGHlinkGHR (See figure 1).

The ligation process removes the 3' stop codons within the GHstop region thus allowing transcription of the full length GHlinkGHR.

The same strategy was employed in order to clone in the full length extracellular domain of GHR incorporating SD100 N and C-terminal domains.

Construction of full length extracellular domain of GHR (GHRflec)

The full length extracellular domain of GHR (SD100 N and C-terminal) was amplified using primers GHRS1ECOR and GHRA835H (see Table 1) following the same PCR protocol as described earlier for the generation of GHstop. The 5'-nucleotide (GHRS1ECOR) contains an EcoRI site and the 3'-nucleotide contains a HindIII site. The PCR reaction produced a band of 762bp (see Figure 7) containing full length extracellular GHR and purified using Qiaquick PCR clean up kit (Qiagen). Both vector, pTrcHisGHlinkGHR and PCR product were subjected to a double restriction digest using EcoRI and HindIII restriction enzymes. The PCR product was cleaned up using QIAquick PCR clean up kits and the digested vector was separated by agarose gel electrophoresis and subsequently purified using the QIAquick gel extraction kit.

Both vector, pTrcHisGHlinkGHR, and PCR product were double digested with EcoRI and HindIII, and cleaned up using QIAquick clean up kits (Qiagen). The digested PCR fragment was then ligated in to the digested pTrcHisGHlinkGHR vector and transformed in to TOPO one shot cells (Invitrogen) by the calcium chloride method. Positive transformants were screened by restriction digest using EcoRI and HindIII and by PCR using primers GHRS1ECOR and GHRA835H. Clones with the correct sized insert were sequenced using GHseqF and the vector specific primer pTrcHis reverse. The new

construct was called pTrcHisGHlinkGHRflec. This can then be used as a template for any future linker inserts.

Cloning GHstop and GHlinkGHR into pJONEX4

pJONEX4 vector (See Figure1) was constructed in order to express inducible proteins that were potentially deleterious to the cell by placing them under the control of a strong repressor of transcription (cI857) and a heat inducible promoter (PLλ). The construction of pJONEX4 has been described elsewhere (Jon R. Sayers and Fritz Eckstein; Nucleic Acid Research, volume19, No15, p4127-4132, 1991).

The PLλ promoter region was cloned into pUC19 in the EcoRI site and engineered so that only one EcoRI site remained downstream of the promoter to produce pJONEX4. Genes wishing to be transcribed can be inserted into the SacI/HindIII region downstream of the PLλ promoter. This vector can be used to transform bacteria which specify a temperature sensitive lambda repressor (cI857), thus at low temperatures, below 30°C, transcription read through is prevented by the presence of the repressor protein. However, at higher temperatures (42°C) induction of protein expression proceeds. The main aim was to construct primers in order to PCR up the full length GHstop and GHlinkGHR from their parent vector pTrcHis-TOPO and subclone these fragments into the SacI/HindIII sites in pJONEX4.

5'-nucleotide, TrcRBSsacF contains an engineered SacI restriction site, a new ribosome binding site and the ATG start codon present in the pTrcHis-TOPO vector. Two 3'-nucleotides will be used to PCR GHstop and GHlinkGHR respectively from their parent vectors, pTrcHis. The 3'-nucleotide, TrcHindrev, contains a HindIII site and will be used to PCR the full length GHstop gene. The other nucleotide, GHRA835H has already been described, and will be used to PCR up GHlinkGHR (see Table 1).

TrcRBSSacIf:

5' gggaaa **gagctc** aaggagaaaataaa atg ggggggttctcatcatcat 3'

SacI RBS START pTrc vector

TrcHindIIIrev:

5'gccaagcttcgaattgaattcg 3'

PCR Method

96°C 2mins

94°C 30sec/54°C 1min/72°C 1min, for 30 cycles

72°C10mins

PCR reaction; Master Mix 1

Plasmid (100ng total)	2µl pTrcHisGHstop	2µl pTrcHisGhlinkGHR	2µl water	2µl water
Forward primer (10mM stock)	2µl TrcRBSsac1	2µl TrcRBSsacI	2µl TrcRBSsac1	2µl TrcRBSsacI
Reverse primer (10mM stock)	2µl TrcHindrev	2µl GHRA835H	2µl TrcHindrev	2µl GHRA835H
DNTP (10mM stock)	2µl	2µl	2µl	2µl
Sterile water	17µl	17µl	17µl	17µl
Total volume	25µl	25µl	25µl	25µl

Master Mix 2 (per reaction)

Expand High Fidelity buffer (plus magnesium, 1.5mM final) (5µl)
Expand High Fidelity polymerase: (0.6µl)
Sterile water: (19.4µl)

Added 25µl Master Mix 2 to Master mix 1 and overlaid with mineral oil.

Both PCR fragments and pJONEX4 vector were subjected to a double restriction digest using SacI/HindIII and purified using the QIAquick clean up kits (Qiagen). The digested PCR fragment was then ligated to the above digested vector and transformed in to E. coli M72 (λ) cells by the method of electroporation. Plasmid mini preps were produced from positive transformants and screened by restriction digest using SacI/HindIII and by PCR using nucleotides TrcRBSsac1 and TrcHindrev for Ghstop and TrcRBSsac1 and GHRA835H for GHlinkGHR. Clones with the correct insert size were then sequenced using GHS1-23, GhseqF, Xpress forward and GHA573not.

Cloning full length IL-6 and gp130 into pTZ18U/pTrcHis-TOPO/pJONEX4 vectors

The IL-6 / gp130 chimeras are provided in a variety of vectors. Cloning into pTZ18U will facilitate *in vitro* mutagenisis and the pJONEX and pTrcHis-TOPO vectors can be used to generate recombinant protein in *E. coli* which can be purified using Nickel columns.

Cloning is into pTrcHis using the TA cloning strategy devised for Ghstop/GHlinkGHR. The chimeras are then subcloned into the pJonex and pTZ18U system using the restriction sites BamH1/HindIII. This would maintain the upstream RBS and Hist6 tag in pJONEX and allow insertion into pTZ18U (they have the same multiple cloning site) for mutagenesis experiments.

The strategy is to TA clone in IL-6 (full length: see sequence below: Figure 1) with the 3'prime nucleotide containing a Not 1 site together with another restriction site: Sal1 (or Xho1). This Sal1 site will thus allow the cloning of the gp130 gene in to the Sal1/HindIII sites (HindIII being in 3'end of the pTrcHis vector). The linker can then be inserted into the Not1/Sal1 sites.

The construct once sequenced is subloned into the pJonex and pTZ18U vectors using BamHI/HindIII.

IL-6 and gp130 are amplified by PCR from IMAGE clones or cDNA from human lymphocytes.

The following primers will be used in TA cloning of the IL-6 sequence as represented in Figure 11 into pTrcHis.

Primers for cloning IL-6 into pTrcHis

Forward (5'nucleotide) PRIMER 1

5' gtacccccagg agaagattcc aaagatgtag 3' (31 mer with 15gc)

Reverse primer (3'nucleotide: NotI/SalI and Stop codons are shown in bold, sequence shown in italics and underlined is insert sequence to keep sequence in frame and as an overhang for NotI/SalI digestion and incorporates the stop codons)

PRIMER 2

5' tgagggctcttcggcaaatg g gcggccgc *tgataa* gtcgac 3' (20 mer with 11gc)

5' cagctg *aatagt* cgccggcg g gtaaacggcttctcgggagt 3'

5' gtcgac *ttatca* gcggccgc c catttgccgaagagccctca 3'(reverse nucleotide)

The next stage is to sub-clone the gp130 full length extracellular domain (322-2112bp; see Figure 12). Clone gp130 into the Sal1/HindIII sites

5

Primers for cloning full length gp130 into pTrcHis-TOPO

Forward primer (5'nucleotide : SalI site shown in bold) PRIMER 3

10 5' gggaaa **gtcgac** gaacttcta gatccatgtg gtt3' (22 mer 9gc)

Reverse primer (HindIII and stop codons shown in bold) PRIMER 4

15 5' ccaaa gtttgct caaggagaaattgaa **tgataa aagctt** gggaaa 3'

5' aaaggg **ttcgaa aatagt** aagttaaagaggaac tcgtttg aaacc3'

20 5' tttccc **aagctt ttatca** ttcaatttctccttg agcaaac tttgg3' (reverse nucleotide)

The step 3 is to ligate in the linker duplex that contain a 5'Not1 site and a 3'Sal1 site.

25

LINKER DUPLEX

G4S4 Not/SalI (5' overhang for Not1 and 3'overhang for SalI are shown in bold) PRIMER 7

30

5'**ggccg**cggtggcggaggtagtggtggcggaggtagcggtggcggaggttctggtggcggaggttcc **g**

G4S4rev/ Not/Sal 1(5' overhang for Not1 and SalI are shown in bold) PRIMER 8

5' **tcgac** ggaacctccgccaccagaacctccgccaccgctacctccgccaccactacctccgccacc **gc** 3'

This produces a full length construct: IL-6/link/gp130. The next step is to carry out cloning of domain deletions of gp130 into the SalI/HindIII sites.

Primers for cloning gp130 D1 deletion in to pTrcHIs-TOPO (SalI/HindIII sites)

forward primer (SalI site shown in bold) PRIMER 5

5' gggaaa **gtcgac** atttcaggcttgcctcca 3'

Reverse primer (HindIII and stop codons shown in bold) PRIMER 4

5' ccaaa gtttgct caaggagaaattgaa **tgataa aagctt** gggaaa 3'

5' aaaggg **ttcgaa aatagt** aagttaaagaggaac tcgtttg aaacc3'

5' tttccc **aagctt ttatca** ttcaatttctccttg agcaaac tttgg3' (reverse nucleotide)

The next step is to clone in gp130 truncation up to 922bp (this deletes domains 1 and 2 from the extracellular region of gp130). Construct IL-6/link/gp130D1

Primers for cloning gp130 (922-2112bp fragment)

Forward primer (SalI site shown in bold) PRIMER 6

5' gggaaa **gtcgac** aatccgccacataatttat 3'

Reverse primer (HindIII and stop codons shown in bold) PRIMER 4

5' ccaaa gtttgct caaggagaaattgaa **tgataa aagctt** gggaaa 3'

5' aaaggg **ttcgaa aatagt** aagttaaagaggaac tcgtttg aaacc3'

5' tttccc **aagctt ttatca** ttcaatttctccttg agcaaac tttgg3' (reverse nucleotide)

Preparation of Electrocompetant M72 (λ) cells

M72 (λ) cells were grown o/n in 50ml LB. 100ml of this o/n culture was then added to 900ml LB and grown at 30°C until OD600 was between 0.5-0.6. Cells were then harvested at 4000rpm, 20 min at room temperature using a Sorval RC-3B centrifuge. The pellet was resuspended and re-centrifuged at 4000rpm, 4°C, 20 minutes in gradually reducing volumes of sterile ice cold 10% (v/v) glycerol of 1000ml, 500ml, 250ml. The pellet was finally resuspended in 1000µl of 10% (v/v) glycerol, divided in to 100µl aliquots, flash frozen in liquid nitrogen and stored at -80°C.

Transformation of M72 cells.

Electrocompetant M72 (λ) cells were defrosted on ice and placed in to an electroporation cuvette (cell width of 0.1cm, Invitrogen) and electroporated at 1.8KV. Positive tranformants were selected for on LB plates supplemented with 100µg/ml ampicillin and grown at 30°C overnight.

Induction of Expression of constructs from pTrcHis-TOPO vectors

Transformed *E. coli* TOP 10 cells were grown overnight at 37°C with shaking at 200rpm in 10ml LB supplemented with ampicillin (100μg/ml final). The next day 5ml of the overnight was used to seed 250ml LB supplemented with ampicillin (100μg/ml final) and grown to an OD600 = 0.6. The culture was then induced with the addition of IPTG to a final concentration of 1mM and the cells grown for a further 5hrs. Induced cells were then harvested by centrifugation at 13000rpm, room temperature and the pellet either frozen or lysed.

Induction of Expression of constructs from pJONEX vectors

Transformed *E. coli* M72 (λ) cells were grown o/n at 30°C with shaking at 2000rpm in LB supplemented with ampicillin (100μg/ml). The next day the o/n culture was used to seed fresh LB and cells were grown until an OD600 of approximately 0.6 was reached. The temperature of the incubator was then adjusted to 42°C and an equal volume of pre-warmed media was added to bring the temperature of the culture up to 42°C. The cells were then grown at 42°C for a further 4-5hrs then harvested.

Purification of induced proteins by immobilised metal affinity chromatography (IMAC)

Induced cell pellets were resuspended in 20mM sodium phosphate buffer, 500mM sodium chloride, pH 7.8 and lysed by the addition of hen egg white lysozyme to a final concentration of 100μg/ml, and left on ice for 15 minutes. The cells suspension was then sonicated by applying three 10 second bursts on a medium intensity setting whilst holding on ice. Insoluble material was then removed by centrifugation at 40000 x g, 4°C for 20 minutes in a RC-3B centrifuge.

The cleared cell lysate was then applied to a Probond resin column (Invitrogen) pre-equilibrated with 20mM sodium phosphate buffer, 500mM NaCl, pH 7.8. The column washed with 20mM sodium phosphate buffer, 500mM sodium chloride, pH 7.8 buffer followed by washing with 20mM sodium phosphate buffer, 500mM

sodium chloride, pH 6.0. Bound protein was eluted by an increasing gradient of 50mM to 500mM imidazole made up in 20mM sodium phosphate buffer, 500mM sodium chloride, pH 6.0 buffer. 1ml fractions were collected and purification monitored by bradford protein assay and SDS-PAGE. Fractions containing proteins of interest were pooled and dialysed against 1000 volumes 20mM sodium phosphate buffer, pH 7.8, for 2, 4 and 6 hours respectively. Dialysed protein was then concentrated (if needed) using an Amicon Centriprep Y-10 column. Dialysed and concentrated samples were then either stored at 4°C or frozen or used directly in a bioassay for growth hormone activity.

Bioassay of rGH and purified growth hormone constructs

Hek293 cells were previously stabily transfected with full-length human growth hormone receptor. Cells were routinely cultured in Dulbeccos MEM/Nutrient F12 medium supplemented with 10% Foetal calf serum, 1% penicillin/streptomycin and 1% L-glutamine. Cells used for the bioassay were first dissociated, counted, then plated at 2x105 cells/ml in growth medium in a 12 well plate and grown o/n at 37°C, 5% CO2. The next day cells were placed in rich medium [2/3 Dulbeccos MEM/F12 nutrient medium, 1/3 Dulbeccos 4.5g/L-glucose, 10% Foetal calf serum, 1% penecillin/streptomycin and 1% L-glutamine] and incubated for 6 hours at 37°C. Transfection with reporter gene constructs was completed using the calcium phosphate transfection system (Life Technologies) according to the manufactures instructions. Cells were left overnight at 37°C, 5% CO2. The next day cells were challenged with recombinant protein from 5-5000ng/ml, made up in starvation medium [Dulbeccos MEM/Nutrient F12 medium supplemented with 1% penicillin/streptomycin, 1% L-glutamine] supplemented with 100ng/µl dexamethasone. Where necessary recombinant wild type GH was mixed with purified GHstop or Chimeric protein in a competition assay. Cells were incubated at 37°C, 5% CO2 for at least 5 hours before assaying for luciferase and ß-galactosidase activity.

Luciferase/ ß-galactosidase assay

The assays were performed according to the manufacturers instructions. Briefly media was aspirated from a 12 well plate and cells lysed with 150µl reporter lysis buffer for 20 minutes at room temperature.

For the ß-galactosidase assay 25µl of each lysate was added to duplicate wells of a 96 well plate and mixed with 75µl assay buffer. The plate was incubated at 37°C until a yellow coloration had developed at which point the plate was read at 420nm. For the luciferase assay, 50µl of the remaining lysate was added to a luminometer cuvette to which was then added 50µl of luciferase substrate. The sample was mixed by vortexing for 10 seconds and fluorescence measured at 15 and 60 second intervals.

The final data was corrected for ß-galactosidase expression by presenting results as a ratio of luciferase:ß-galactosidase activity measured. Figure 17 shows data generated from a reporter gene assay using purified GH stop and GHlinkGHR.

Western blotting

Samples from purification's were routinely analysed for growth hormone expression by first separating samples by 12% (v/v) SDS-PAGE under either reducing or non-reducing conditions and transferring to PVDF membrane. The membrane was then blocked in 4%(w/v) milk protein in PBS, supplemented with 0.05% (v/v) Tween 20 (PBS-T). The membrane was then probed with anti-growth hormone (10A7, mouse IgG1) at 1/2000 dilution in 1% (w/v) milk protein in PBS-T. After brief washing the membrane was probed with Sheep anti mouse-HRP (Amersham) at 1/5000 dilution in 1%(w/v) milk protein in PBS-T. After extensive washing with PBS-T, specific protein bands were visualised using ECL western blot detection reagents (Amersham). Figure 16 shows a western blot of induced proteins expressed either in the pTrcHis-TOPO of pJONEX vector systems.

Radioimmunoassay for growth hormone

The human growth hormone assay was performed using the NETRIA human growth hormone IRMA assay which uses a rabbit polyclonal and a labelled monoclonal antibody.

Table 1: RIA results for induced lysates of Ghstop and GHlinkGHR

Sample	Value (mU/L)
Ghstop induced cell lysate	583
GHlinkGHR induced cell lysate	504
Non-transfected cell lysate	42

Testing metabolic clearance rate in vivo

Sprague-Dawley rats are anaesthetised and cannulae implanted in femoral and jugular veins. Two days later GH or chimera is administered by intravenous or subcutaneous injection. Blood samples are collected via the femoral cannula and chimera levels measured by radio-immunoassay (see table 2). Pharmacokinetic parameters are estimated using available computer programs fitting hormone concentration against time.

Activation of GH signalling, measured as luciferase activity, by GH, negative control purification and Chi 1A2 (GH fused to GHR)

A number of chimeric constructs were made. The partially purified chimera was prepared from transformed XL blue *E. coli*. Protein from untransformed XL blue *E. coli* was purified over nickel columns and used as a negative control to detect any non-specific agonist or antagonist action. All purified proteins were stored in glycerol.

The negative control and Chimera 1A2 were incubated with and without GH.

Figure 19 shows results of bioassay comparing the induction of a Stat5 reporter (luciferase activity) by growth hormone (GH), negative control (XL blue), and partially purified antagonist (Chimera 1A2).

The graph shows the expected dose-response to GH. Incubation with negative control showed no induction of luciferase activity but at high concentration partially inhibited the bioassay (this may be an effect of the increased glycerol concentration). At 500 ng/ml Chimera 1 A2 appeared to completely block GH signalling.

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CLAIMS

1. A binding agent comprising a first part capable of binding a ligand binding domain of a receptor linked to a second part comprising a receptor binding domain wherein said binding agent modulates the activity of the receptor.

2. A binding agent according to claim 1 wherein the binding agent antagonises the activity of the receptor.

3. A binding agent according to claim 1 wherein the binding agent agonises the activity of the receptor.

4. A binding agent according to any of claims 1 to 3 wherein the first part comprises a cytokine or the binding domain of a cytokine.

5. A binding agent according to claim 4 wherein the cytokine or the binding is selected from the following: growth hormone; leptin; erythropoietin; prolactin; TNF, interleukins (IL), IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-10, IL-11; the p35 subunit of IL-12, IL-13, IL-15; granulocyte colony stimulating factor (G-CSF); granulocyte macrophage colony stimulating factor (GM-CSF); ciliary neurotrophic factor (CNTF); cardiotrophin-1 (CT-1); leukemia inhibitory factor (LIF); oncostatin M (OSM); interferon, IFNα and IFNγ.

6. A binding agent according to claim 4 or claim 5 wherein the second part comprises at least part of the cognate receptor of the cytokine or a part of an associated receptor.

7. A binding agent according to claim 5 or claim 6 wherein the first part is GH.

8. A binding agent according to any of claims 4 to 7 wherein the second part is one extracellular domain of GHR.

9. A binding agent according to claim 8 wherein the second part is the C-terminal SD-100 domain of GHR.

10. A binding agent according to claim 5 or claim 6 wherein the first part is IL-6 or a binding domain of IL-6 and the second part is a part of the IL-6 receptor or gp 130.

11. A binding agent according to any of claims 1 to 10 wherein the binding agent is a fusion protein.

12. A binding agent according to any of claims 1 to 11 wherein the first part is linked by a linker to the second part.

13. A binding agent according to claim 12 wherein the first part is GH and linkage is made between a residue close to the C-terminus of the GH molecule and a residue close to the N-terminus of the GHR.

14. A binding agent according to claim 13 wherein linkage is made between a residue close to the C-terminus of the GH molecule and a residue close to the N-terminal of the N-terminal of the C-terminal SD-100.

15. A binding agent according to claim 14 wherein linkage is made at any of residues 126-128 of the N-terminus of the C-terminal SD-100 of the GHR.

16. A binding agent according to claim 15 wherein linkage is made at residue 127 of the N-terminus of the C-terminal SD-100.

17. A binding agent according to any of claims 12 to 16 wherein the linker is a nucleic acid, a peptide nucleic acid or a chemical crosslinker.

18. A binding agent according to claim 17 wherein the linker is a peptide..

19. A binding agent according to claim 18 wherein the linker is a polypeptide which comprises 5 to 30 amino acid residues.

20. A binding agent according to claim 19 wherein the linker comprises 10 to 20 amino acid residues.

21. A binding agent according to any of claims 18 to 20 wherein the linker comprises at least one copy of the peptide:

Gly Gly Gly Gly Ser (hereinafter referred to as "Gly4Ser").

22. A binding agent according to claim 21 wherein the linker comprises two copies of the Gly4Ser linker.

23. A binding agent according to claim 21 wherein the linker comprises three copies of the Gly4Ser linker.

24. A binding agent according to claim 21 wherein the linker comprises four copies of the Gly4Ser linker.

25. A nucleic acid molecule comprising a nucleic acid sequence which encodes a binding agent according to any of Claims 1 to 24 selected from the group consisting of:

i) the group comprising Figures 4, 5, 8, 9 and 21

ii) nucleic acids which hybridise to the sequences of (i) above and which have receptor antagonising activity; and

iii) nucleic acid sequences which are degenerate as a result of the genetic code to the sequences defined in (i) and (ii) above.

26. A nucleic acid according to Claim 25 which hybridises under stringent hybridisation conditions to the sequences represented in Figs 4, 5, 8, 9, or 21.

27. A polypeptide encoded by the nucleic acid according to claim 25 or 26.

28. A polypeptide according to claim 27 which is modified by deletion, addition or substitution of at least one amino acid residue.

29. A vector comprising the DNA molecule of any of claims 25 to 26.

30. A vector according to claim 29 wherein the vector is adapted for recombinant expression.

31. A vector according to claim 29 or claim 30 wherein said vector is an expression vector adapted for prokaryotic gene expression.

32. A vector according to claim 29 or claim 30 wherein said vector is an expression vector adapted for eukaryotic gene expression.

33. A vector according to any of claims 29 to 32 wherein said vector is provided with and therefore said binding agent includes a secretion signal to facilitate purification of said binding agent.

34. A cell transformed/transfected with the nucleic acid according to any of claims 25 to 26 or the vector according to any of claims 29 to 33.

35. A cell according to claim 34, wherein the cell is a eukaryotic cell.

36. A cell according to claim 35 wherein said cell is selected from the group consisting of: fungal cell; insect cell; amphibian cell; plant cell or mammalian cell.

37. A cell according to claim 34 wherein said cell is a prokaryotic cell.

38. A cell according to claim 37 wherein said cell is *Escherchia coli.*

39. A pharmaceutical composition comprising the binding agent according to any of claims 1-24.

40. A pharmaceutical composition according to claim 39 wherein said composition further comprises a carrier, excipient and/or a diluent.

41. The use of a binding agent according to any of claims 1-24 for the manufacture of a medicament for use in the treatment of acromegaly; gigantism; GH deficiency; Turners syndrome; renal failure; osteoporosis; diabetes mellitus; cancer; obesity; insulin resistance; hyperlipidaemia; hypertension; anaemia;autoimmune and infectious disease; inflammatory disorders including rheumatoid arthritis (IL-6 chimera)

42. A method to prepare a binding agent polypeptide according to any of the preceding claims comprising:

i) growing a cell transformed/transfected with a nucleic acid according to any of claims 25 to 26 or the vector of any of claims 29 to 33 in conditions conducive to the manufacture of said polypeptide; and

ii) purifying said polypeptide from said cell, or its growth environment.

43. A method of treating a human or animal subject comprising administering an effective amount of the agent according to any of claims 1-24 or the pharmaceutical composition of any of claims 39 or 40 to said subject.

44. A nucleic acid comprising the nucleic acid sequence represented in Figure 4.

45. A nucleic acid comprising the nucleic acid sequence represented in Figure 5.

46. A nucleic acid comprising the nucleic acid sequence represented in Figure 8.

47. A nucleic acid comprising the nucleic acid sequence represented in Figure 9.

48. A nucleic acid comprising the nucleic acid sequence represented in Figure 21.

49. A polypeptide comprising the amino acid sequence of any of claims 6, 11 13 or 22.

50. A method for the manufacture of a binding agent according to any of claims 1-24 which provides a binding agent which has reduced systemic clearance comprising linking a ligand binding domain of a receptor to a receptor binding domain.

51. A method according to claim 50 wherein said binding agent is at least 70kDa.

52. A method according to claim 51 wherein said binding agent is between 70 and 80kDa.

53. A method according to claim 52 wherein said binding agent is greater than 80kDa.

Fig 1a

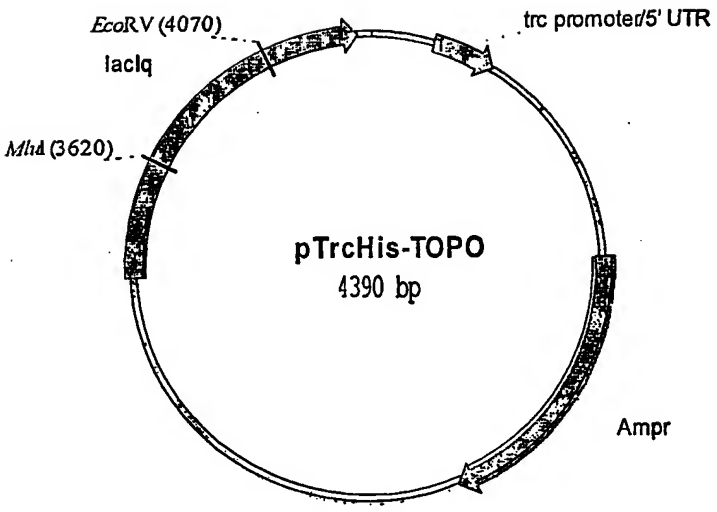

Fig 1b

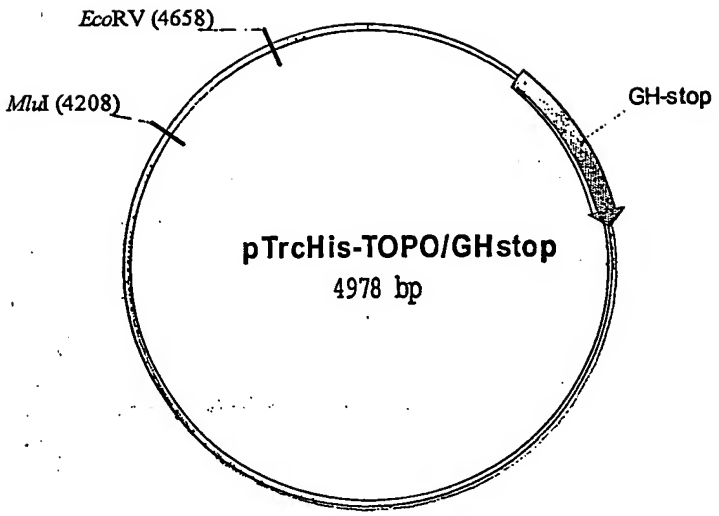

Fig 1c

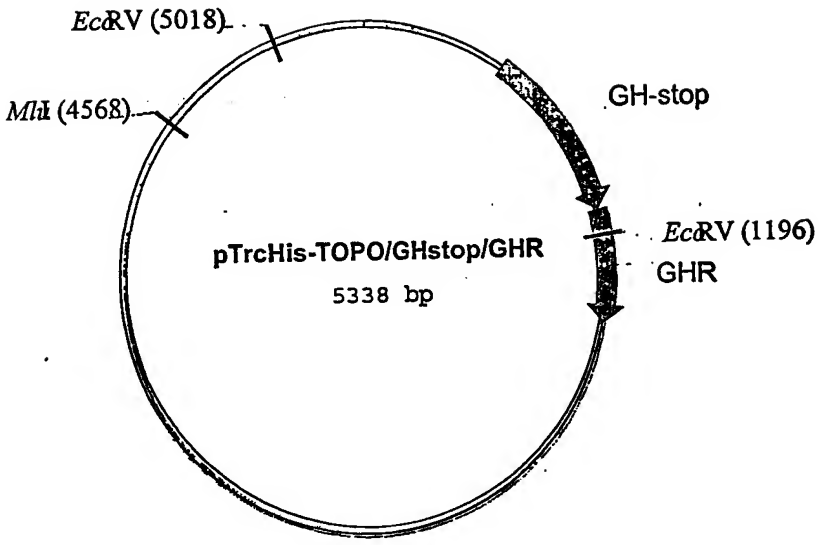

Fig 1d

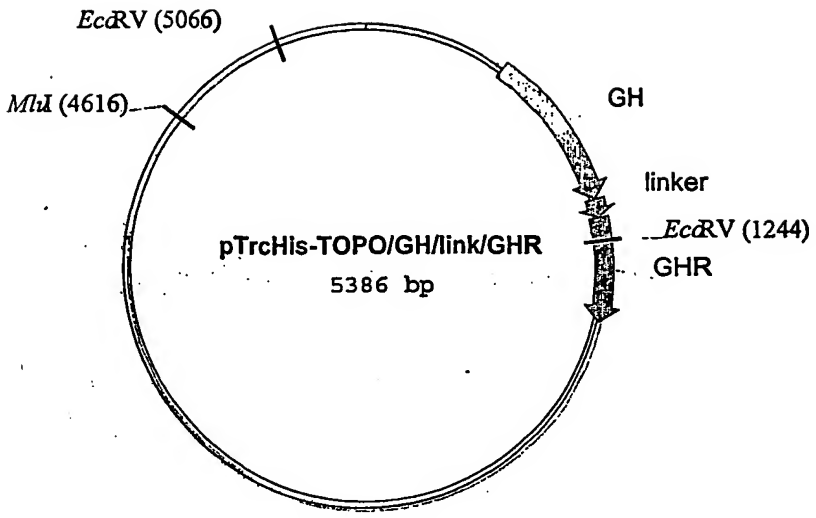

Fig 1e

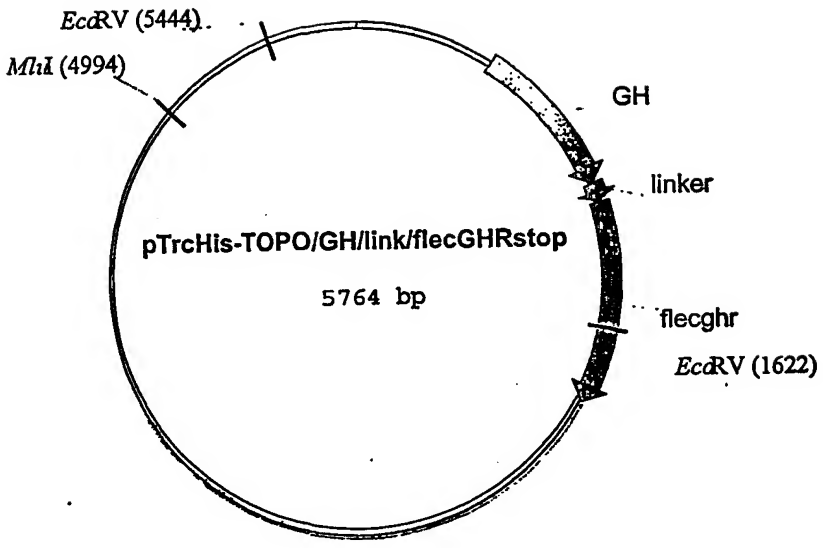

Fig 1f

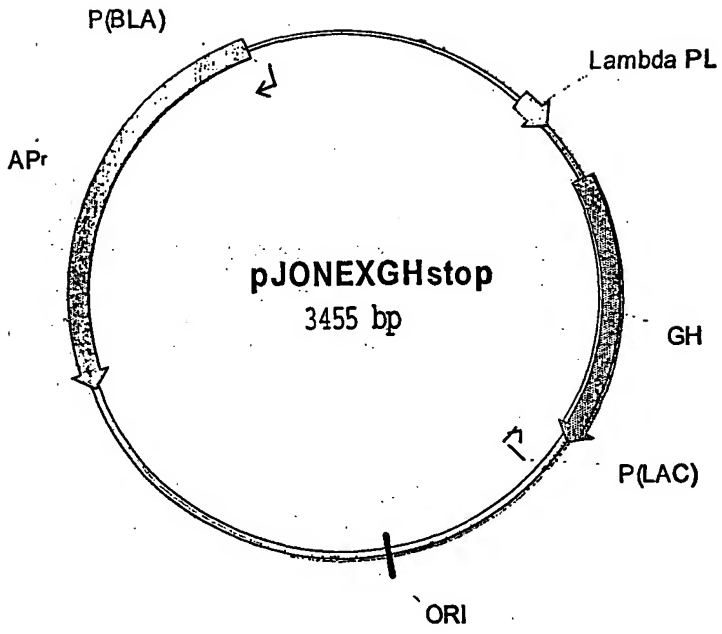

Fig 1g

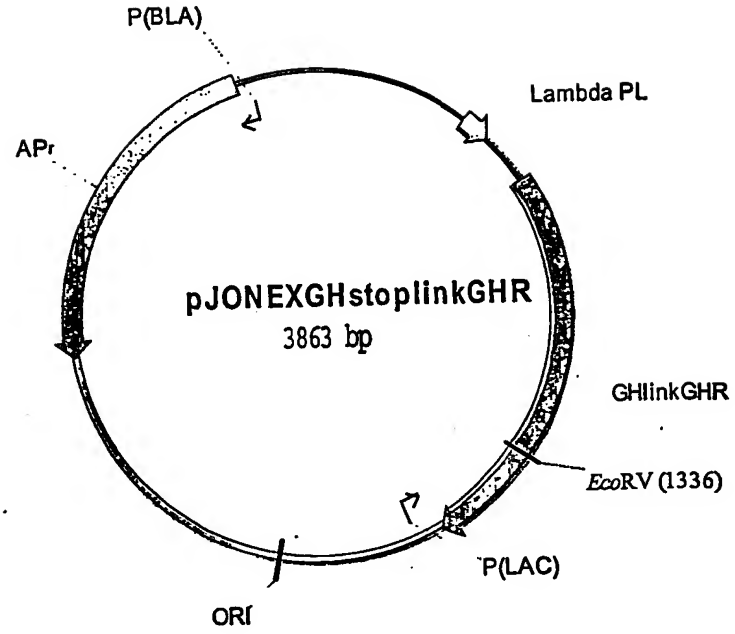

Figure 2:

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tccgacaccctccaacagggaggaaacacaacagaaatccaacctagagctgctccgcatctccctgctgctcatccagtcgt
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aggacatggacaaggtcgagacattcctgcgcatcgtgcagtgccgctctgtggagggcagctgtggcttc<u>ggcggccgc</u>*tg*
ataa

Figure 3:

gggaaa**gaattc**gaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatg
cagatatccaagtgagatgggaagcaccacgcaatgcagatattcagaaaggatggatggttctggagtatgaacttcaataca
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Figure 4

GHstop

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gagtttgaagaagcctatatcccaaaggaacagaagtattcattcctgcagaaccccccagacctccctctgtttctcagagtctat
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aggacatggacaaggtcgagacattcctgcgcatcgtgcagtgccgctctgtggagggcagctgtggcttc**ggcggccgc***tg*
ataa

GHR

aagggc**gaattc**gaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatg
cagatatccaagtgagatgggaagcaccacgcaatgcagatattcagaaaggatggatggttctggagtatgaacttcaataca
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atgagccaatttacatgtgaagaagatttcta*ctgataa***aagctt**

Figure 5:

Growth Hormone:

ttcccaaccattcccttatccaggctttttgacaacgctatgctccgcgcccatcgtctgcaccagctggcctttgacacctaccag
gagtttgaagaagcctatatcccaaaggaacagaagtattcattcctgcagaacccccagacctccctctgtttctcagagtctat
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aggacatggacaaggtcgagacattcctgcgcatcgtgcagtgccgctctgtggagggcagctgtggcttcggcggccgc

Linker

ggtggcggaggtagtggtggcggaggtagcggtggcggaggttctggtggcggaggttcc

Growth hormone receptor

gaattcgaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatgcagatat
ccaagtgagatgggaagcaccacgcaatgcagatattcagaaaggatggatggttctggagtatgaacttcaatacaaagaag
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aatttacatgtgaagaagatttctac*tgataa*aagctt

Figure 6

fptiplsrlfdnaslrahrlhqlafdtyqefeeayipkeqkysflqnpqtslcfsesiptpsnreetqqksnlellrislliqswle
pvqflrsvfanslvygasdsnvydllkdleegiqtlmgrledgsprtgqifkqtyskfdtnshnddallknygllycfrkdmd
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adiqkgwmvleyelqykevnetkwkmmdpilttsvpvyslkvdkeyevrvrskqrnsgnygefsevlyvtlpqmsqf
tceedfy**kl

Figure 7

gggaaag**aattc**ttttctggaagtgaggccacagcagctatccttagcagagcaccctggagtctgcaaagtgttaatccagg
cctaaagacaaattcttctaaggagcctaaattcaccaagtgccgttcacctgagcgagagacttttcatgccactggacagat
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agttcagtgaggtgctctatgtaacacttcctcagatgagccaatttacatgtgaagaagatttctac*tgataa***aagctt**

Figure 8

Growth hormone

ttcccaaccattcccttatccaggctttttgacaacgctatgctccgcgcccatcgtctgcaccagctggcctttgacacctaccag
gagtttgaagaagcctatatcccaaaggaacagaagtattcattcctgcagaacccccagacctccctctgtttctcagagtctat
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aggacatggacaaggtcgagacattcctgcgcatcgtgcagtgccgctctgtggagggcagctgtggcttc**ggcggccgc**

Linker

ggtggcggaggtagtggtggcggaggtagcggtggcggaggttctggtggcggaggttcc

Growth hormone receptor :N-terminal SD100

Gaattcttttctggaagtgaggccacagcagctatccttagcagagcaccctggagtctgcaaagtgttaatccaggcctaaa
gacaaattcttctaaggagcctaaattcaccaagtgccgttcacctgagcgagagacttttcatgccactggacagatgaggttc
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C-terminal SD100

Gaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatgcagatatccaag
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atgtgaagaagatttctac*tgataa***aagctt**

Figure 9

Growth hormone

gggaaa**gagctc**aaggagaaaataaa*atg*gggggttctcatcatcatcatcatggtatggctagcatgactggtggaca
gcaaatgggtcgggatctgtacgacgatgacgataaggatccaacccttttcccaaccattcccttatccaggctttttgacaacg
ctatgctccgcgcccatcgtctgcaccagctggcctttgacacctaccaggagtttgaagaagcctatatcccaaaggaacaga
agtattcattcctgcagaacccccagacctccctctgtttctcagagtctattccgacaccctccaacagggaggaaacacaaca
gaaatccaacctagagctgctccgcatctccctgctgctcatccagtcgtggctggagcccgtgcagttcctcaggagtgtcttc
gccaacagcctggtgtacggcgcctctgacagcaacgtctatgacctcctaaaggacctagaggaaggcatccaaacgctga
tggggaggctggaagatggcagcccccggactgggcagatcttcaagcagacctacagcaagttcgacacaaactcacaca
acgatgacgcactactcaagaactacgggctgctctactgcttcaggaaggacatggacaaggtcgagacattcctgcgcatc
gtgcagtgccgctctgtggagggcagctgtggcttc**ggcggccgc**

Linker

ggtggcggaggtagtggtggcggaggtagcggtggcggaggttctggtggcggaggttcc

Growth hormone receptor

gaattcgaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatgcagatat
ccaagtgagatgggaagcaccacgcaatgcagatattcagaaaggatggatggttctggagtatgaacttcaatacaaagaag
taaatgaaactaaatggaaaatgatggaccctatattgacaacatcagttccagtgtactcattgaaagtggataaggaatatgaa
gtgcgtgtgagatccaaacaacgaaactctggaaattatggcgagttcagtgaggtgctctatgtaacacttcctcagatgagcc
aatttacatgtgaagaagatttctac*tgataa***aagctt**gggaaa

Figure 10

gagctcaaggagaaaataaa*atg*gggggttctcatcatcatcatcatggtatggctagcatgactggtggacagcaaat
gggtcgggatctgtacgacgatgacgataaggatccaacccttttcccaaccattcccttatccaggctttttgacaacgctatgc
tccgcgcccatcgtctgcaccagctggcctttgacacctaccaggagtttgaagaagcctatatcccaaaggaacagaagtatt
cattcctgcagaacccccagacctccctctgtttctcagagtctattccgacaccctccaacagggaggaaacacaacagaaat
ccaacctagagctgctccgcatctccctgctgctcatccagtcgtggctggagcccgtgcagttcctcaggagtgtcttcgccaa
cagcctggtgtacggcgcctctgacagcaacgtctatgacctcctaaaggacctagaggaaggcatccaaacgctgatgggg
aggctggaagatggcagcccccggactgggcagatcttcaagcagacctacagcaagttcgacacaaactcacacaacgat
gacgcactactcaagaactacgggctgctctactgcttcaggaaggacatggacaaggtcgagacattcctgcgcatcgtgca
gtgccgctctgtggagggcagctgtggcttc**ggcggccgc***tgataa*aagggc**gaattcaattcgaa**gcttggc

Figure 11

A

gagctc<u>**aaggagaaaataaa**</u>*at*ggggggttctcatcatcatcatcatggtatggctagcatgactggtggacagcaaat
gggtcgggatctgtacgacgatgacgataaggatccaacccttttcccaaccattcccttatccaggctttttgacaacgctatgc
tccgcgcccatcgtctgcaccagctggcctttgacacctaccaggagtttgaagaagcctatatcccaaaggaacagaagtatt
cattcctgcagaacccccagacctccctctgtttctcagagtctattccgacaccctccaacagggaggaaacacaacagaaat
ccaacctagagctgctccgcatctccctgctgctcatccagtcgtggctggagcccgtgcagttcctcaggagtgtcttcgccaa
cagcctggtgtacggcgcctctgacagcaacgtctatgacctcctaaaggacctagaggaaggcatccaaacgctgatgggg
aggctggaagatggcagcccccggactgggcagatcttcaagcagacctacagcaagttcgacacaaactcacacaacgat
gacgcactactcaagaactacgggctgctctactgcttcaggaaggacatggacaaggtcgagacattcctgcgcatcgtgca
gtgccgctctgtggagggcagctgtggcttc**ggcggccgc***tgataa*aagggc**gaattcaattcgaag**cttggc

B

```
  1    VPPGEDSK DVAAPHRQPL TSSERIDKQI RYILDGISAL RKETCNKSNM
 51  CESSKEALAE NNLNLPKMAE KDGCFQSGFN EETCLVKIIT GLLEFEVYLE
101  YLQNRFESSE EQARAVQMST KVLIQFLQKK AKNLDAITTP DPTTNASLLT
151  KLQAQNQWLQ DMTTHLILRS FKEFLQSSLR ALRQMGGR** VDKG
```

Figure 12

gaacttcta gatccatgtg gttatatcag tcctgaatct ccagttgtac aacttcattc taatttcact gcagtttgtg tgctaaagga aaaatgtatg gattattttc atgtaaatgc taattacatt gtctggaaaa caaaccattt tactattcct aaggagcaat atactatcat aaacagaaca gcatccagtg tcacctttac agatatagct tcattaaata ttcagctcac ttgcaacatt cttacattcg gacagcttga acagaatgtt tatggaatca caataatttc aggcttgcct ccagaaaaac ctaaaaattt gagttgcatt gtgaacgagg ggaagaaaat gaggtgtgag tgggatggtg gaagggaaac acacttggag acaaacttca ctttaaaatc tgaatgggca acacacaagt ttgctgattg caaagcaaaa cgtgacaccc ccacctcatg cactgttgat tattctactg tgtattttgt caacattgaa gtctgggtag aagcagagaa tgcccttggg aaggttacat cagatcatat caattttgat cctgtatata aagtgaagcc caatccgcca cataatttat cagtgatcaa ctcagaggaa ctgtctagta tcttaaaatt gacatggacc aacccaagta ttaagagtgt tataatacta aaatataaca ttcaatatag gaccaaagat gcctcaactt ggagccagat tcctcctgaa gacacagcat ccacccgatc ttcattcact gtccaagacc ttaaaccttt tacagaatat gtgtttagga ttcgctgtat gaaggaagat ggtaagggat actggagtga ctggagtgaa gaagcaagtg ggatcaccta tgaagataga ccatctaaag caccaagttt ctggtataaa atagatccat cccatactca aggctacaga actgtacaac tcgtgtggaa gacattgcct ccttttgaag ccaatggaaa aatcttggat tatgaagtga ctctcacaag atggaaatca catttacaaa attacacagt taatgccaca aaactgacag taaatctcac aaatgatcgc tatctagcaa ccctaacagt aagaaatctt gttggcaaat cagatgcagc tgttttaact atccctgcct gtgactttca agctactcac cctgtaatgg atcttaaagc attccccaaa gataacatgc tttgggtgga atggactact ccaagggaat ctgtaaagaa atatatactt gagtggtgtg tgttatcaga taaagcaccc tgtatcacag actggcaaca agaagatggt accgtgcatc gcacctattt aagagggaac ttagcagaga gcaaatgcta tttgataaca gttactccag tatatgctga tggaccagga agccctgaat ccataaaggc ataccttaaa caagctccac cttccaaagg acctactgtt cggacaaaaa aagtagggaa aaacgaagct gtcttagagt gggaccaact tcctgttgat gttcagaatg gatttatcag aaattatact atattttata gaaccatcat tggaaatgaa actgctgtga atgtggattc ttcccacaca gaatatacat tgtcctcttt gactagtgac acattgtaca tggtacgaat ggcagcatac acagatgaag gtgggaagga tggtccagaa ttcacttttа ctaccccaaa gtttgct caa ggagaaattg aa

Figure 13

```
  1    VPPGEDSKDV AAPHRQPLTS SERIDKQIRY ILDGISALRK ETCNKSNMCE
 51  SSKEALAENN LNLPKMAEKD GCFQSGFNEE TCLVKIITGL LEFEVYLEYL
101  QNRFESSEEQ ARAVQMSTKV LIQFLQKKAK NLDAITTPDP TTNASLLTKL
151  QAQNQWLQDM TTHLILRSFK EFLQSSLRAL RQMGGRGGGG SGGGGSGGGG
201  SGGGGSVDEL LDPCGYISPE SPVVQLHSNF TAVCVLKEKC MDYFHVNANY
251  IVWKTNHFTI PKEQYTIINR TASSVTFTDI ASLNIQLTCN ILTFGQLEQN
301  VYGITIISGL PPEKPKNLSC IVNEGKKMRC EWDGGRETHL ETNFTLKSEW
351  ATHKFADCKA KRDTPTSCTV DYSTVYFVNI EVWVEAENAL GKVTSDHINF
401  DPVYKVKPNP PHNLSVINSE ELSSILKLTW TNPSIKSVII LKYNIQYRTK
451  DASTWSQIPP EDTASTRSSF TVQDLKPFTE YVFRIRCMKE DGKGYWSDWS
501  EEASGITYED RPSKAPSFWY KIDPSHTQGY RTVQLVWKTL PPFEANGKIL
551  DYEVTLTRWK SHLQNYTVNA TKLTVNLTND RYLATLTVRN LVGKSDAAVL
601  TIPACDFQAT HPVMDLKAFP KDNMLWVEWT TPRESVKKYI LEWCVLSDKA
651  PCITDWQQED GTVHRTYLRG NLAESKCYLI TVTPVYADGP GSPESIKAYL
701  KQAPPSKGPT VRTKKVGKNE AVLEWDQLPV DVQNGFIRNY TIFYRTIIGN
751  ETAVNVDSSH TEYTLSSLTS DTLYMVRMAA YTDEGGKDGP EFTFTTPKFA
801  QGEIE**KL
```

Figure 14

atttcaggcttgcctccagaaaaacctaaaaatttgagttgcattgtgaacgaggggaagaaaatgaggtgtgagtgggatggt
ggaagggaaacacacttggagacaaacttcactttaaaatctgaatgggcaacacacaagtttgctgattgcaaagcaaaacgt
gacacccccacctcatgcactgttgattattctactgtgtattttgtcaacattgaagtctgggtagaagcagagaatgcccttggg
aaggttacatcagatcatatcaattttgatcctgtatataaagtgaagcccaatccgccacataatttatcagtgatcaactcagag
gaactgtctagtatcttaaaattgacatggaccaacccaagtattaagagtgttataatactaaaatataacattcaatataggacca
aagatgcctcaacttggagccagattcctcctgaagacacagcatccacccgatcttcattcactgtccaagaccttaaacctttt
acagaatatgtgtttaggattcgctgtatgaaggaagatggtaagggatactggagtgactggagtgaagaagcaagtgggat
cacctatgaagatagaccatctaaagcaccaagtttctggtataaaatagatccatcccatactcaaggctacagaactgtacaa
ctcgtgtggaagacattgcctccttttgaagccaatggaaaaatcttggattatgaagtgactctcacaagatggaaatcacattta
caaaattacacagttaatgccacaaaactgacagtaaatctcacaaatgatcgctatctagcaaccctaacagtaagaaatcttgt
tggcaaatcagatgcagctgttttaactatccctgcctgtgactttcaagctactcaccctgtaatggatcttaaagcattccccaaa
gataacatgctttgggtggaatggactactccaagggaatctgtaaagaaatatatacttgagtggtgtgtgttatcagataaagc
accctgtatcacagactggcaacaagaagatggtaccgtgcatcgcacctatttaagagggaacttagcagagagcaaatgct
atttgataacagttactccagtatatgctgatggaccaggaagccctgaatccataaaggcataccttaaacaagctccaccttcc
aaaggacctactgttcggacaaaaaaagtagggaaaaacgaagctgtcttagagtgggaccaacttcctgttgatgttcagaat
ggatttatcagaaattatactatattttatagaaccatcattggaaatgaaactgctgtgaatgtggattcttcccacacagaatatac
attgtcctctttgactagtgacacattgtacatggtacgaatggcagcatacacagatgaaggtgggaaggatggtccagaattc
acttttactaccccaaagtttgct caaggagaaattgaa

Figure 15

aatccgccacataatttatcagtgatcaactcagaggaactgtctagtatcttaaaattgacatggaccaacccaagtattaagagt
gttataatactaaaatataacattcaatataggaccaaagatgcctcaacttggagccagattcctcctgaagacacagcatccac
ccgatcttcattcactgtccaagaccttaaacctttacagaatatgtgtttaggattcgctgtatgaaggaagatggtaagggata
ctggagtgactggagtgaagaagcaagtgggatcacctatgaagatagaccatctaaagcaccaagtttctggtataaaataga
tccatcccatactcaaggctacagaactgtacaactcgtgtggaagacattgcctccttttgaagccaatggaaaaatcttggatt
atgaagtgactctcacaagatggaaatcacatttacaaaattacacagttaatgccacaaaactgacagtaaatctcacaaatgat
cgctatctagcaaccctaacagtaagaaatcttgttggcaaatcagatgcagctgttttaactatccctgcctgtgactttcaagcta
ctcaccctgtaatggatcttaaagcattccccaaagataacatgctttgggtggaatggactactccaagggaatctgtaaagaa
atatatacttgagtggtgtgtgttatcagataaagcaccctgtatcacagactggcaacaagaagatggtaccgtgcatcgcacc
tatttaagagggaacttagcagagagcaaatgctatttgataacagttactccagtatatgctgatggaccaggaagccctgaat
ccataaaggcataccttaaacaagctccaccttccaaaggacctactgttcggacaaaaaaagtagggaaaaacgaagctgtc
ttagagtgggaccaacttcctgttgatgttcagaatggatttatcagaaattatactatattttatagaaccatcattggaaatgaaac
tgctgtgaatgtggattcttcccacacagaatatacattgtcctctttgactagtgacacattgtacatggtacgaatggcagcatac
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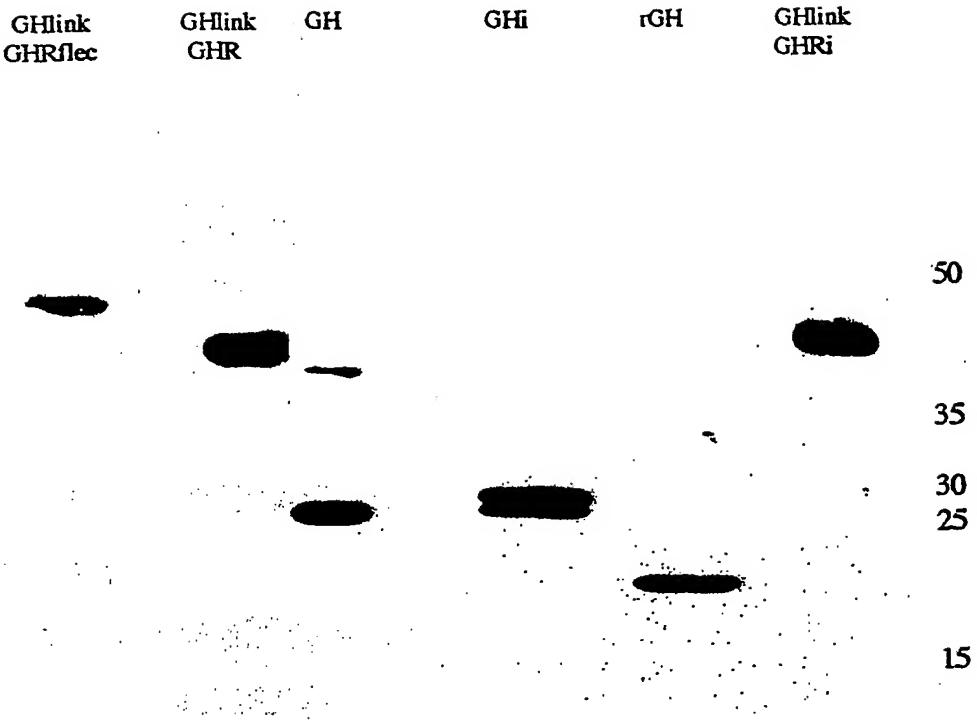
FIGURE 16.



Sample description:

GHlinkGHRflec: pTrcHisGHlinkGHRflec, contains full length extracellular domain of growth hormone receptor (IPTG induced).

GHlinkGHR: pTrcHisGhlinkGHR, contains only C-terminal SD100 of growth hormone receptor (IPTG induced)

GH: pTrcHisGHstop, contains full length growth hormone (IPTG induced)

GHi: pJonexGHstop, contains full length GH (heat induced)

RGH: purified recombinant human growth hormone

GHlinkGHRi: pJonexGHlinkGHR, contains full length GhlinkGHR (heat induced)

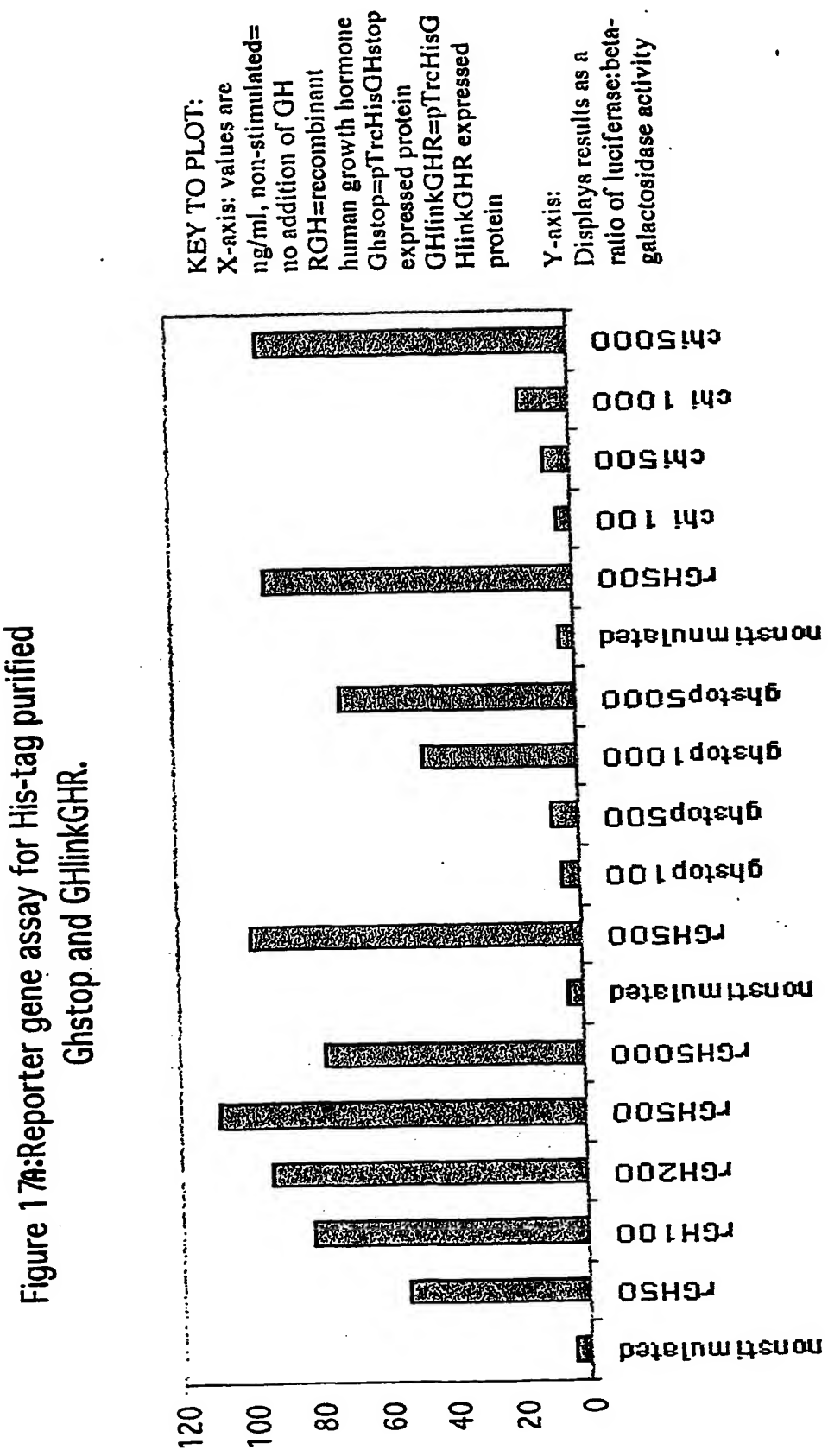
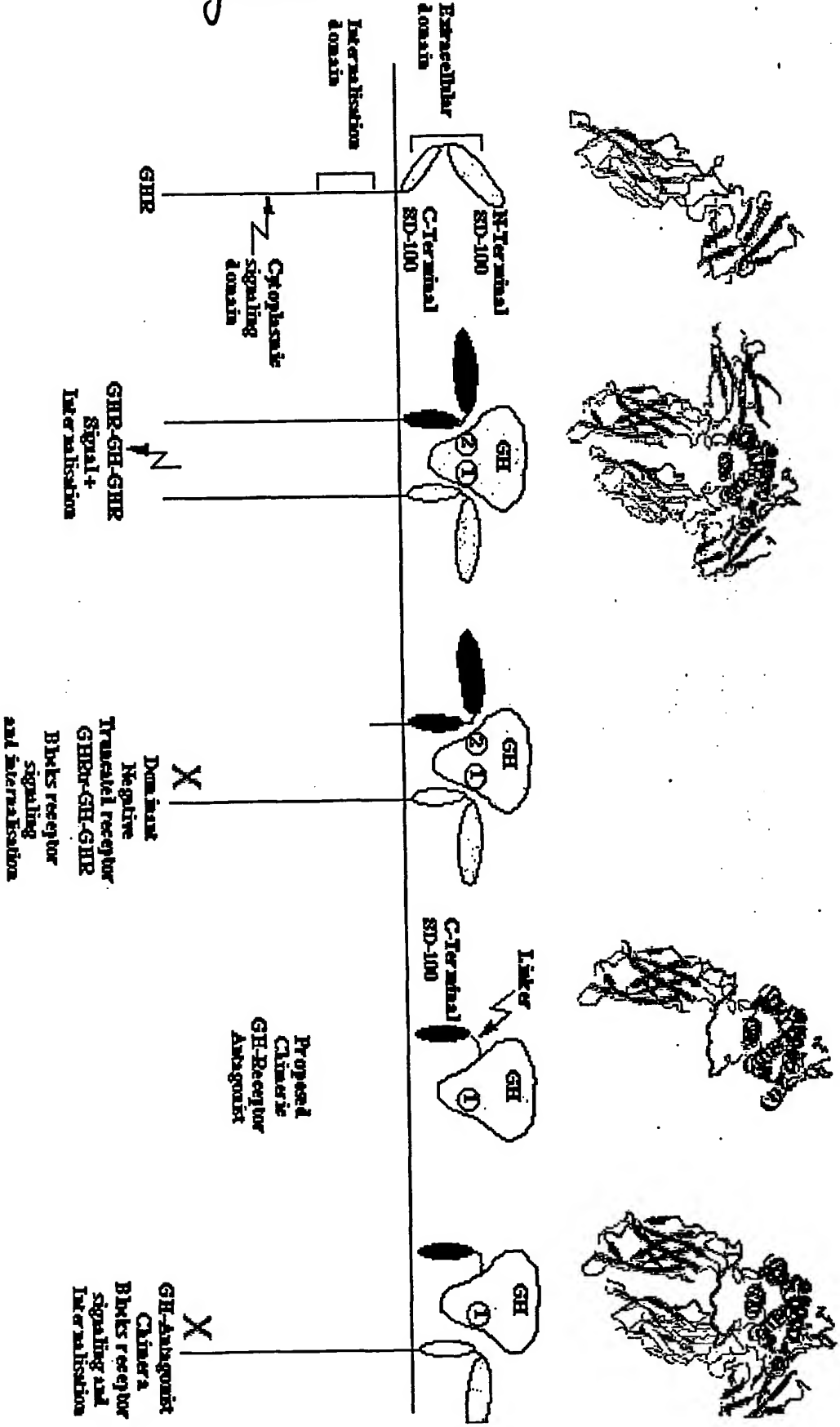


Figure 17A:Reporter gene assay for His-tag purified Ghstop and GHlinkGHR.

Figure 17B: Reporter gene assay: Table of results obtained for His-tag purified Ghstop and GHlinkGHR

Sample	Activity ratio	Standard error	Fold induction
Non stimulated	4.54	0.3	1
rGH 50	53.73	1.46	11.8
rGH 100	82.08	3.3	18.1
rGH 200	93.65	5.57	20.6
rGH 500	108.54	5.02	23.9
rGH 5000	76.93	13.37	16.9
Non stimulated	4.61	0.6	1
rGh 50	98.61	7.9	21.4
Ghstop 100	5.36	0.05	1.2
Ghstop 500	8.44	1.3	1.8
Ghstop 1000	45.92	0.56	10
Ghstop 5000	71.24	6.89	15.4
Non stimulated	4.38	0.91	1
rGH 100	92.76	0.92	21.1
Chi 500	8.12	2.82	1.85
Chi 1000	15.18	16	3.46
Chi 5000			

Figure 18 :



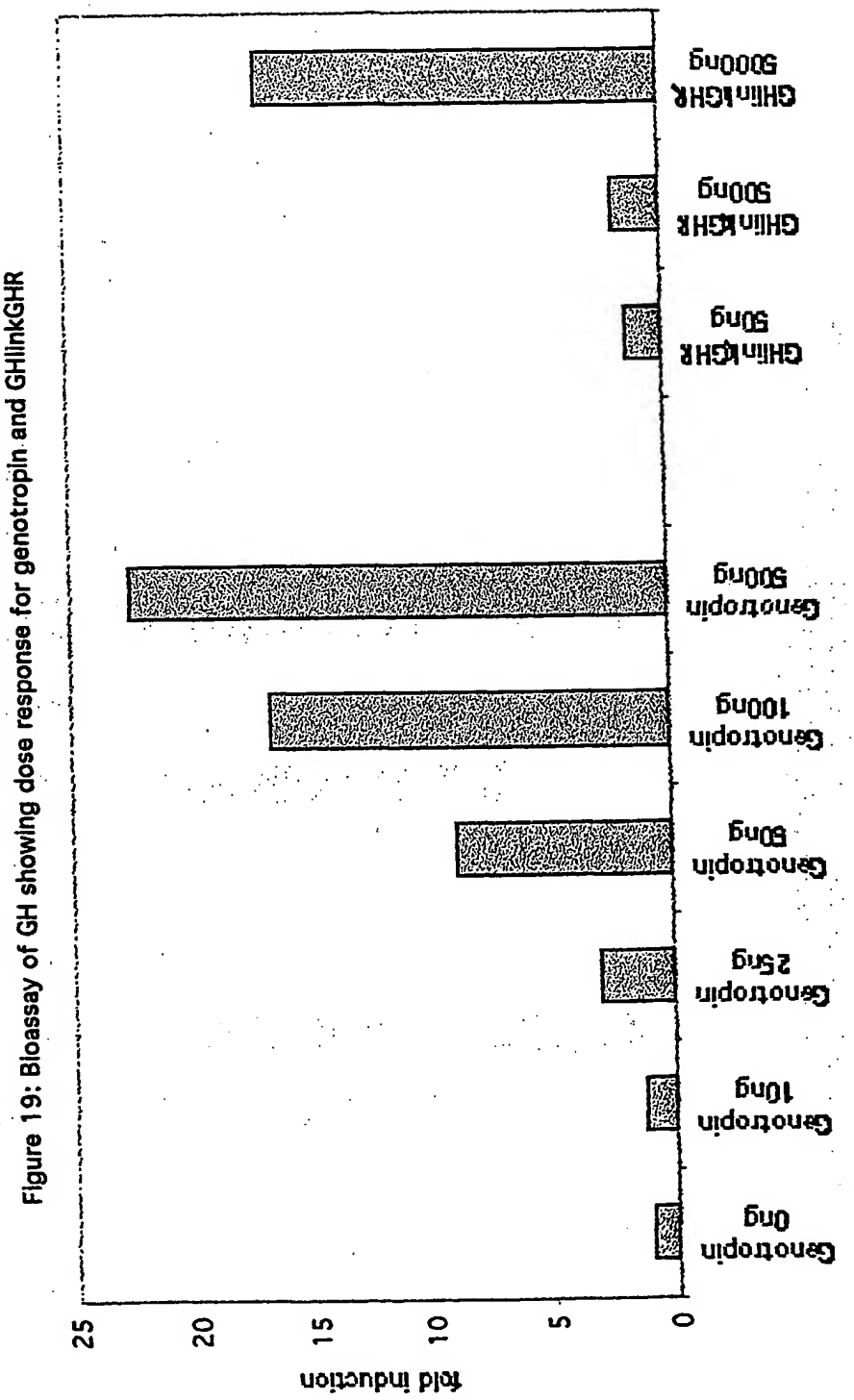


Figure 19: Bioassay of GH showing dose response for genotropin and GHlinkGHR

FIGURE 20;
Activation of Gh signalling, measured as luciferase activity, Gh, negative control purification and Chl1A2 (Gh fused to GHR)

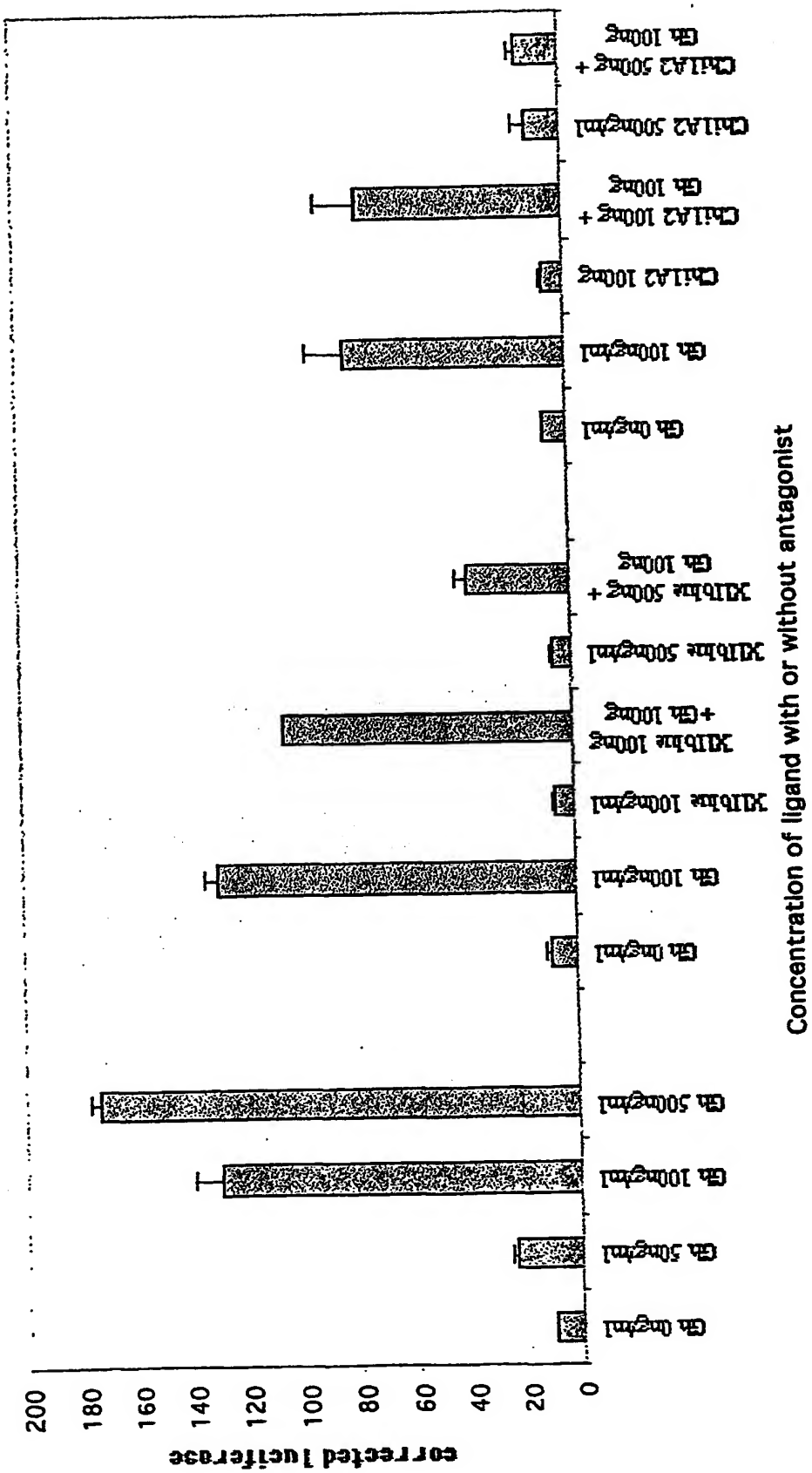


Figure 21: The chimera 1A2 was generated using the phagemid method to remove the linker sequence from GHlinkGHR and generate a fusion protein of the C-terminus of growth hormone directly the N-terminus of the GHR SD100. The DNA sequence is given below (GH in bold and GHR SD100 in italics)

ttcccaaccattcccttatccaggctttttgacaacgctatgctccgcgcccatcgtctgcaccagctggcctttgacacctaccaggagtttga
agaagcctatatcccaaaggaacagaagtattcattcctgcagaaccccccagacctccctctgtttctcagagtctattccgacaccctccaa
cagggaggaaacacaacagaaatccaacctagagctgctccgcatctccctgctgctcatccagtcgtggctggagcccgtgcagttcctca
ggagtgtcttcgccaacagcctggtgtacggcgcctctgacagcaacgtctatgacctcctaaaggacctagaggaaggcatccaaacgct
gatggggaggctggaagatggcagcccccggactgggcagatcttcaagcagacctacagcaagttcgacacaaactcacacaacgatg
acgcactactcaagaactacgggctgctctactgcttcaggaaggacatggacaaggtcgagacattcctgcgcatcgtgcagtgccgctct
gtggagggcagctgtggcttc*gaaatagtgcaaccagatccacccattgccctcaactggactttactgaacgtcagtttaactgggattcatgc*
agatatccaagtgagatgggaagcaccacgcaatgcagatattcagaaaggatggatggttctggagtatgaacttcaatacaaagaagtaa
atgaaactaaatggaaaatgatggaccctatattgacaacatcagttccagtgtactcattgaaagtggataaggaatatgaagtgcgtgtgaga
tccaaacaacgaaactctggaaattatggcgagttcagtgaggtgctctatgtaacacttcctcagatgagccaatttacatgtgaagaagatttct
actgataaaagctt

Figure 22: Protein sequence of Chi 1A2 (311 amino acids)

Fptiplsrlfdnaslrahrlhqlafdtyqefeeayipkeqkysflqnpqtslcfsesiptpsnreetqqksnlellrislliqswlepvqflrsv
fanslvygasdsnvydllkdleegiqtlmgrledgsprtgqifkqtyskfdtnshnddallknygllycfrkdmdkvetflrivqcrsveg
scgf*eivqpdppialrwtllrvsltgihadiqvrweaprnadiqkgwmvleyelqykevnetkwkmmdpilttsvpvyslkvdkeyevrvrskq*
*rnsgnygefsevlyvtlpqmsqftceedfy***kl

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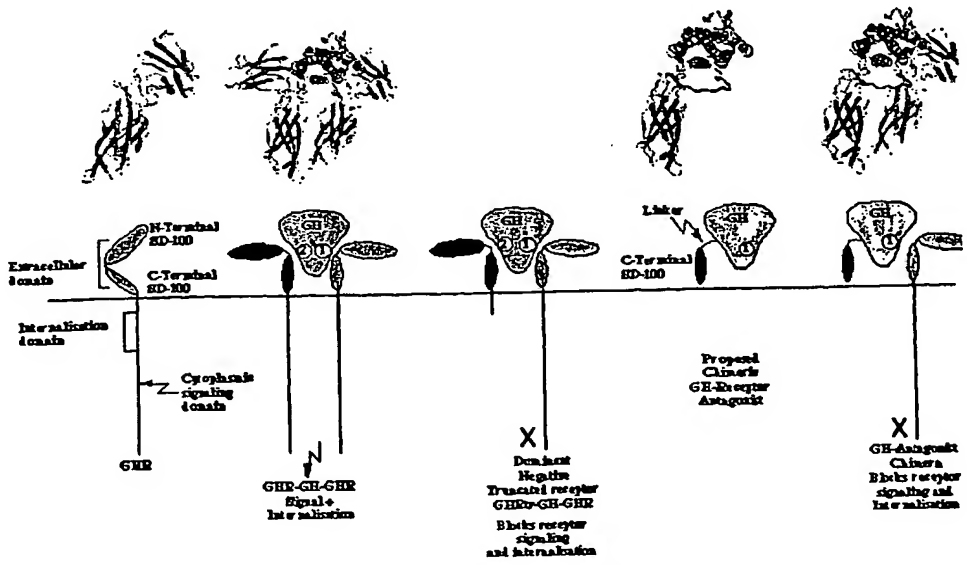
(54) Title: BINDING AGENTS: CHIMERIC LIGAND/RECEPTOR PROTEINS



(57) Abstract: This invention relates to agents which bind to cell surface receptors; methods to manufacture said agents; therapeutic compositions comprising said agents; and screening methods to identify novel agents.

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C07K14/61 C07K14/715 C07K14/72 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
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Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
BIOSIS, EPO-Internal, SEQUENCE SEARCH, MEDLINE, PAJ, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 97 32891 A (ANGEWANDTE GENTECHNOLOGIE SYST ;ROSE JOHN STEFAN (DE)) 12 September 1997 (1997-09-12) page 6 figures 1,2	1-6, 10-12, 17-23, 50-53
X	WO 99 02552 A (KOLLET ORIT ;LAPIDOT TSVEE (IL); CHEBATH JUDITH (IL); REVEL MICHEL) 21 January 1999 (1999-01-21) page 7, line 16 -page 8 page 28 -page 30; example 1 figure 11 page 54 -page 60; claims 1-37 page 32; example 2	1-6, 10-12, 17-20, 50-53

-/—

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Date of the actual completion of the international search	Date of mailing of the international search report
7 February 2002	21 02 2002

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Blanco Urgoiti, B

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Int: ...nal Application No
PCT/GB 01/02645

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4 857 637 A (MARTIN JR DAVID W ET AL) 15 August 1989 (1989-08-15) figure 2	44-47
X	WO 86 05804 A (NORDISK GENTOFTE) 9 October 1986 (1986-10-09) figure 1	44-46
X	WO 90 05185 A (UNIV LIEGE) 17 May 1990 (1990-05-17) figure 3	44-46
A	ROSS R J M ET AL: "A short isoform of the human growth hormone receptor functions as a dominant negative inhibitor of the full-length receptor and generates large amounts of binding protein." MOLECULAR ENDOCRINOLOGY, vol. 11, no. 3, 1997, pages 265-273, XP002181691 ISSN: 0888-8809 cited in the application	
A	WO 00 18932 A (STAHL NEIL ;REGENERON PHARMA (US); YANCOPOULOS GEORGE D (US)) 6 April 2000 (2000-04-06)	
A	WO 92 21029 A (GENENTECH INC) 26 November 1992 (1992-11-26)	
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A	MORAN P ET AL: "A NONFUNCTIONAL SEQUENCE CONVERTED TO A SIGNAL FOR GLYCOPHOSPHATIDYLINOSITOL MEMBRANE ANCHOR ATTACHMENT" JOURNAL OF CELL BIOLOGY, vol. 115, no. 2, 1991, pages 329-336, XP001031102 ISSN: 0021-9525	
A	YIYING ZHANG ET AL: "POSITIONAL CLONING OF THE MOUSE OBESE GENE AND ITS HUMAN HOMOLOGUE" NATURE, MACMILLAN JOURNALS LTD. LONDON, GB, vol. 372, no. 6505, 1 December 1994 (1994-12-01), pages 425-432, XP000602062 ISSN: 0028-0836	

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INTERNATIONAL SEARCH REPORT

International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	EP 0 741 187 A (HOFFMANN LA ROCHE) 6 November 1996 (1996-11-06)	
A	HIBI M ET AL: "MOLECULAR CLONING AND EXPRESSION OF AN IL-6 SIGNAL TRANSDUCER, GP130" CELL, CELL PRESS, CAMBRIDGE, NA, US, vol. 63, 21 December 1990 (1990-12-21), pages 1149-1157, XP002931740 ISSN: 0092-8674	

INTERNATIONAL SEARCH REPORT

ational application No.
PCT/GB 01/02645

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Although claim 43 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [X] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

(1-4,7-10,13-16,25-38,42,44-48) complete; (5,6,11,12,17-24,39-41,43,49-53) partially

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[X] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. Claims: (1-4,7-9,13-16,25-38,42,44-48) complete; (5,6,11, 12,17-24,39-41,43,49-53) partially

A binding agent comprising the binding domain of a ligand linked to a receptor binding domain, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent comprising at least part of a cytokine; said binding agent comprising GH and the extracellular domain of GHR or the C-terminal SD-100 domain of GHR; said binding agent being a fusion protein;said binding agent wherein both domains are linked by a linker; said binding agent wherein the linkage is made close to the C-terminus of GH and the N-terminus of GHR, or of the C-terminal SD-100 domain of GHR; wherein the linker comprises at least one copy of the peptide "Gly4Ser"; a nucleic acid comprising figures 4,5,8,9 or 21, derived polypeptides, vectors, host cells, pharmaceutical compositions, uses in the manufacture of medicaments, methods of preparation, methods of treatment.

2. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of leptin linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

3. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of erythropoietin linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

4. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of prolactin linked to the binding domain of an associated

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

5. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of TNF linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

6. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 2 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

7. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 3 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

8. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 4 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

9. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 5 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

10. Claims: (5,6,11,12,17-24,39-41,43, 49-53) partially; 10 (complete)

A binding agent comprising at least the binding domain of interleukin 6 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said receptor being the IL-6 receptor or gp 130; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

11. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 7 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

12. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 9 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

in the manufacture of medicaments, and methods of preparation.

13. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 10 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

14. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 11 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

15. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the p35 subunit of interleukin 12 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

16. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 13 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

17. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interleukin 15 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

18. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of granulocyte colony stimulating factor (G-CSF) linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

19. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of granulocyte macrophage colony stimulating factor (GM-CSF) linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

20. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of ciliary neurotrophic factor (CNTF) linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

21. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

A binding agent comprising at least the binding domain of cardioptrophin-1 linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

22. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of leukemia inhibitory factor (LIF) linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

23. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of oncostatin M (OSM) linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

24. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interferon alpha linked to the binding domain of an associated receptor, wherein the binding agent agonises or antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

25. Claims: (5,6,11,12,17-24,39-41,43,49-53) partially

A binding agent comprising at least the binding domain of interferon gamma linked to the binding domain of an associated receptor, wherein the binding agent agonises or

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

antagonises the activity of the receptor; said binding agent being a fusion protein; said binding agent wherein both domains are linked by a linker; said linker comprises at least one copy of the peptide "Gly4Ser"; pharmaceutical compositions, uses in the manufacture of medicaments, and methods of preparation.

INTERNATIONAL SEARCH REPORT

International Application No
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